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OXIDIZED FLAVOR IN MILK. VIII. THE EFFECT OF THE DEGREE OF SATURATION OF FAT IN THE RATION OF THE COW UPON THE IODINE NUMBER OF THE BUTTER FAT AND THE SUSCEPTIBILITY OF THE MILK TO METAL-INDUCED OXIDIZED FLAVOR

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Feed was one of the e[illegible] influence the susceptibility of milk to oxidized flav[illegible] reported that oleaginous (oxidized) flavor in mil[illegible]uring the winter, and that green feed as well as fre[illegible]rable amounts of reducing substances which tended to prevent oxidized flavor from developing. Stebnitz and Sommer (8) studied the effect of the composition of butter fat on its susceptibility toward oxidation. They found the stability of a fat toward oxidation bore an inverse relationship to the degree of unsaturation of the fat, with linoleic acid content rather than oleic acid the principal governing factor. When cows received grass as part of their ration, the butter fat became less saturated and more susceptible to oxidation. It appeared that protective substances prevented the development of oxidized flavor. Dean and Hilditch (3) found that when cows went on grass there was an abrupt increase in the oleic and linoleic acids and a parallel reduction of butyric and stearic acids in the butter fat.

Prewitt and Parfitt (7) found that rations containing soybean oil, either as such or in the unprocessed beans, have a tendency to produce milk which upon holding developed less intense oxidized flavor than did milk from cows fed other rations. Corbett and Tracy (2) studied the effect of adding cocoanut oil and corn oil to the ration and found that changing the degree of saturation of the fat had no effect on the development or occurrence of oxidized flavors. Gould, Fox, and Trout (4) found no relationship between the susceptibility of milk to copper-induced oxidized flavor and the lecithin content of the milk.

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Although evidence has been submitted (1, 9, 10) that oxidized flavor is not the result of the direct oxidation of the fat itself, it is possible that the degree of saturation of the fat may play a part since milk contains a delicate oxidation-reduction system. The degree of saturation of the fat may affect this system.

In order to obtain information on this point the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, eleven cows whose milks were susceptible to oxidized flavor when contaminated with copper, were selected and placed on a ration low in fat. The ration consisted of a grain mixture of 300 pounds oats, 300 pounds barley, 300 pounds wheat bran, 15 pounds salt, and 10 pounds bone meal, with brown alfalfa hay and beet pulp as roughage.

Samples of milk were collected from each cow each morning and pasteurized in bottles. Following pasteurization the samples were divided into two lots and one lot was contaminated with copper at the rate of none, 0.5 p.p.m., 1.0 p.p.m., and 1.5 p.p.m. of copper added from a solution of copper sulphate. These samples were then stored in ice water for three days, after which they were scored by at least three judges familiar with oxidized flavor. The milk from Lot 2 was composited for two days and churned. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear liquid butter oil was decanted into a clean dry jar, care being taken not to pour off any water or curd collected in the base of the tube. The samples then were ready for iodine number determination. All iodine numbers were determined in duplicate by the Hanus method. Two samples were obtained each week from each cow.

After a preliminary period in which the normal variations in flavor and iodine number from day to day were determined, the cows were divided into two groups, group one to receive the oil supplements, while group two acted as a control. Each cow in group one received a supplement of one pound of oil per day in addition to the ration which they were already receiving. Four types of oil were fed. Cocoanut oil, which supplied a relatively large proportion of the saturated, shorter-chain fatty acids, was fed to cows No. 447 and 452. Crude soybean oil, supplying a high percentage of longer-chain unsaturated fatty acids and a small percentage of lecithin, was fed to cows No. 462 and 463. Refined soybean oil, similar to the crude product except that the lecithin had been almost entirely removed, was fed to cows No. 477 and 478. Hydrogenated soybean oil, a refined soybean oil which had been hydrogenated to give long-chain highly saturated fatty acids, was fed to cows No. 480 and 491. This experimental feeding was continued for six weeks, after which time reversals in feeding were made. The results of

this experiment are shown in table 1. An examination of the averages obtained during this period of feeding as compared with the preliminary period shows, except in one instance, that as the iodine number of the butter fat increases the intensity of the oxidized flavor increases, and as the iodine number decreases the intensity of the oxidized flavor decreases. The oxidized flavor values recorded in this table were obtained by averaging the flavor values from all three copper concentrations for the two days. This gave flavor values comparable to the iodine absorption numbers of the composite fat samples for the same period.

During this period it was found that the crude soybean oil was not as high in natural lecithin (phosphatides) as we had hoped to obtain so a supply of expeller soybean oil was obtained and substituted for the crude soybean oil in the reversal trials. The chief difference between the expeller oil

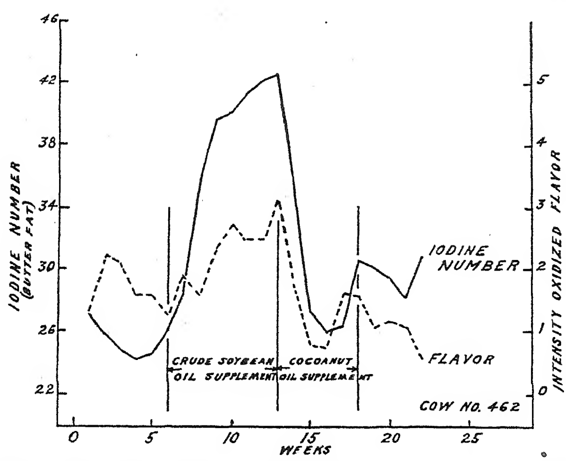

FIG. 1. Effect of fat in the ration on the development of oxidized flavor in milk.

and the crude soybean oil is that the expeller oil is higher in lecithin (phosphatides) content. In making the reversals the change was made so that each individual of any given group received a different type of oil. The effects of this reversal on both the iodine number and the flavor of the milk are shown in the latter part of table 1. These results show quite clearly the effect of the oil supplement on the iodine number of the resulting butter fat. With the exception of the hydrogenated soybean oil the iodine number of the butter fat tended to parallel the iodine number of the fat supplement. In this case the cow apparently desaturated the fat and the resulting butter fat had a slightly higher iodine number than was expected. The relationship between iodine number of the butter fat and the development of oxidized flavor does not readily appear from an examination of table 1. However, when these data are plotted in graph form (figures 1, 2, 3 and 4) it appears evident that as the iodine number increases the intensity of the oxidized

The effect of oil supplements to the ration on the iodine number of the butter fat and on the development of metal-induced oxidized flavor

Preliminary Feeding

	Cow number															
	447		452		462		463		477		478		480		491	
	Iodine number	Flavor*	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor
	28.86	1.00	26.06	1.00	26.90	1.50	27.89	0.83	26.40	0.33	30.36	0.50	28.72	0.66	30.00	3.66
	30.91	0.66	26.24	2.17	27.76	1.17	28.85	1.84	26.95	1.33	29.90	0.66	29.20	1.50	31.55	2.50
	27.55	1.00	25.28	1.17	25.76	2.33	26.92	1.66	26.69	1.50	28.86	1.00	27.83	1.50	25.03	2.66
	25.37	0.66	24.01	2.66	25.91	2.17	27.43	2.66	24.98	1.84	27.65	0.84	27.54	2.66	27.43	3.17
	28.52	0.84	23.70	1.50	25.75	2.00	26.82	3.00	24.93	0.66	27.63	1.00	26.87	2.00	26.74	2.00
	25.70	2.00	22.23	1.66	24.07	2.17	25.44	2.00	23.24	1.17	25.40	1.17	26.74	3.66	27.01	3.17
	23.95	2.00	23.14	2.50	24.81	1.84	26.00	2.66	23.79	2.00	26.47	1.66	27.47	4.33	27.91	3.33
	22.91	0.66	23.10	2.33	23.64	1.33	25.32	1.84	23.87	1.33	26.00	1.50	26.65	3.00	26.22	3.17
	23.40	1.33	23.14	2.17	24.39	1.66	26.26	2.84	23.48	0.66	26.94	1.50	27.38	3.84	27.73	4.17
	26.74	1.50	24.63	2.84	24.93	1.50	26.68	3.84	24.19	1.84	28.67	2.33	27.23	3.50	28.78	4.00
	24.10	1.33	24.14	1.50	26.70	1.33	26.97	2.84	24.93	1.17	28.72	1.66	27.34	2.84	28.01	2.00
	23.56	1.00	24.22	1.66	25.49	1.17	25.77	2.17	24.32	1.00	26.98	1.33	27.58	3.33	29.14	3.33
	24.38	1.17	25.23	2.84	27.19	2.00	27.90	3.50	26.20	1.17	30.13	2.00	27.12	3.33	32.29	2.84
rage	25.84	1.17	24.22	2.00	25.64	1.71	26.79	2.44	24.92	1.23	27.98	1.32	27.51	2.78	28.30	3.08

Oil Supplement

	Cocoanut oil				Crude soybean oil				Refined soybean oil				Hydrogenated soybean oil			
	22.18†	1.84†	21.06†	1.17†	29.79†	1.84†	41.26†	3.84†	36.77†	4.33†	38.53†	3.66†	29.45†	2.00†	30.34†	2.33†
	21.65	0.33	21.48	1.84	35.25	2.00	41.31	4.17	40.94	3.84	40.58	3.00	29.61	2.66	28.92	2.17
	23.72	1.17	22.33	0.17	36.30	1.17	41.66	3.84	39.15	3.66	41.60	2.33	31.02	2.17	33.75	1.50
	22.61	0.00	22.13	0.33	38.62	2.00	44.92	3.66	40.30	3.50	43.42	1.84	33.49	1.17	33.94	1.00
	23.52	0.17	22.40	2.00	40.67	2.66	34.78	3.50	41.50	4.00	45.35	2.50	32.03	1.84	32.15	1.00
	24.44	0.17	23.91	0.50	40.10	2.84	48.29	4.17	43.50	2.66	48.93	2.50	33.41	2.00	31.31	1.00
	24.06	0.00	24.53	0.00	39.77	2.66	49.69	4.33	44.22	1.00	46.90	3.66	35.49	1.66	33.57	1.66
	22.52	0.00	23.77	1.33	41.10	3.00	50.31	2.84	42.10	3.84	49.35	3.50	32.33	2.33	31.61	3.17
	23.99	0.17	24.28	0.50	41.64	2.17	52.06	3.33	46.01	3.17	50.77	4.66	33.74	1.84	34.22	3.00
	24.47	0.33	24.00	0.33	42.53	2.00	52.87	3.17	46.45	4.17	51.55	3.66	33.22	3.00	32.77	3.00
	23.97	0.50	23.40	0.33	41.61	3.17	50.73	3.33	44.27	3.66	50.27	2.33	34.62	3.84	33.61	2.66
	23.86	0.33	23.55	2.00	42.03	3.00	49.17	3.33	42.76	3.33	49.76	2.17	34.48	3.50	33.00	3.66
	22.06	1.33	23.05	1.50	42.91	3.33	49.40	3.00	44.40	3.66	48.19	2.00	34.11	3.84	32.08	2.17
rage	23.31	0.49	23.07	0.92	39.41	2.45	46.65	3.58	42.49	3.45	46.55	2.91	32.85	2.45	32.41	2.18

TABLE 1—(*Continued*)

Oil Reversal

	Cow number															
	447		452		462		463		477		478		480		491	
	Iodine number	Flavor*	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor	Iodine number	Flavor
	Expeller soy-bean oil		Refined soy-bean oil		Cocoanut oil		Hydrogenated soybean oil		Cocoanut oil		Hydrogenated soybean oil		Expeller soy-bean oil		Refined soy-bean oil	
	26.53†	1.00†	28.92†	2.50†	40.31†	2.50†	44.10†	4.33†	37.12†	4.33†	48.01†	1.66†	40.07†	1.84†	38.09†	3.84†
	40.03	0.84	40.78	1.84	28.91	0.84	34.94	1.84	27.89	1.00	41.52	0.50	49.62	3.66	48.96	3.17
	41.42	1.17	40.22	1.66	26.79	0.66	31.96	2.17	27.48	0.66	35.42	1.33	47.17	3.66	49.48	3.17
	41.61	0.66	43.16	1.33	28.03	1.00	32.22	2.66	25.15	0.33	35.18	1.00	50.98	3.66	51.50	3.50
	45.94	2.50	43.84	1.66	25.70	0.84	31.86	1.00	26.60	1.00	33.64	0.66	50.99	2.33	49.12	3.66
	43.99	1.84	44.96	2.33	26.50	0.66	30.02	2.66	24.97	0.50	32.27	2.66	52.01	2.33	51.12	3.66
	42.52	2.66	43.40	2.00	26.28	1.17	28.19	2.00	25.71	0.66	32.89	1.50	45.90	2.33	49.38	3.66
	36.28	2.00	42.86	1.66	26.55	2.17	35.69	1.66	25.82	0.50	32.24	1.50	48.31	3.66	47.53	2.84
'age	39.79	1.58	41.02	1.87	28.63	1.23	33.62	2.29	27.59	1.12	36.40	1.35	48.13	2.93	48.15	3.43
	Readjustment Period															
	32.79†	1.50†	33.61†	2.33†	30.28†	1.50†	lost	lost	28.92†	1.00†	31.50†	1.00†	36.74†	3.00†	35.51†	2.17†
	28.30	1.00	29.06	2.66	30.89	1.66	29.82	2.84	28.24	1.50	30.58	2.00	34.76	3.17	37.94	2.50
	26.25	1.84	28.79	2.17	30.77	1.66	30.18	1.50	28.25	2.17	30.91	1.00	33.40	3.00	32.02	2.17
	27.81	1.00	28.58	3.17	29.58	0.50	29.53	2.66	30.38	1.66	28.93	1.50	31.29	0.84	32.10	3.00
	26.67	1.00	28.13	1.66	29.84	1.17	30.38	1.66	27.14	1.33	30.42	0.84	31.12	2.66	sold	sold
	26.19	1.50	28.38	2.66	29.25	1.17	28.88	2.17	26.30	0.33	31.07	0.84	31.00	2.66		
	25.98	1.33	28.04	1.84	28.88	2.17	29.35	2.66	28.91	0.17	31.93	1.00	33.60	2.50		
	25.39	0.00	26.57	1.33	27.77	0.00	28.93	2.17	25.84	1.17	30.88	0.84	32.21	2.00		
	26.55	0.50	28.37	0.33	30.01	0.84	29.37	2.84	28.91	0.17	31.93	1.00	33.60	2.50		
	29.68	0.50	32.80	0.84	31.95	0.33	33.23	1.84	34.92	0.17	35.80	1.33	38.07	1.50		
'age	27.56	1.02	29.23	1.90	29.92	1.10	29.96	2.26	28.78	0.97	31.40	1.14	33.58	2.38	34.39	2.46

* Oxidized flavor intensity as reported in previous work. JOUR. DAIRY SCIENCE 22: 31. (1939).

† Transition values.

flavor increases. In figure 4 this relationship is uncertain due to wide flavor fluctuations. When hydrogenated soybean oil is supplemented to the ration the iodine number of the butter fat increases. This probably was due to the ability of the cow to desaturate the saturated fat. This same phenomenon

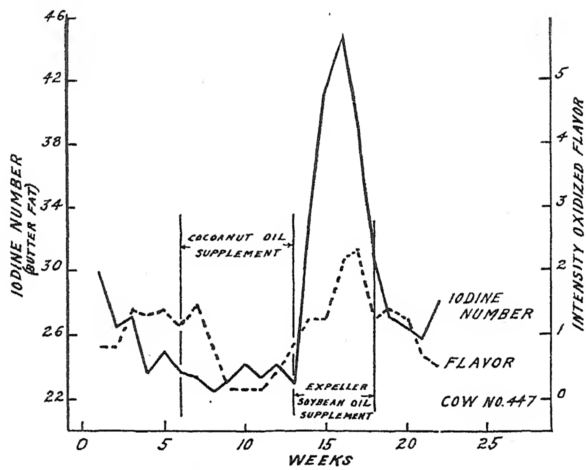

FIG. 2. Effect of fat in the ration on the development of oxidized flavor in milk.

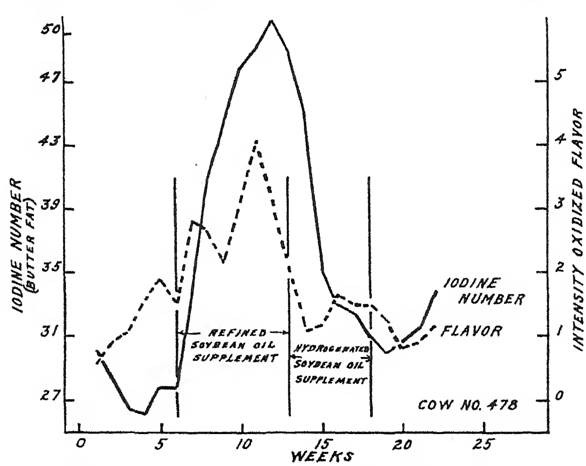

FIG. 3. Effect of fat in the ration on the development of oxidized flavor in milk.

occurred in the case of cow No. 480 on this supplement. Apparently it is the iodine number of the fat in the ration and not the degree of saturation of the butter fat that influences the susceptibility of the milk to metal-induced oxidized flavor.

The relationship between the iodine number of the oil supplement in the ration and the iodine number of the butter fat is shown in table 2.

The cocoanut oil supplement which was high in short-chain fatty acids

and had an iodine number of 11.2, gave butter fat with an average iodine number of 24.2 as compared with 28.1 as the average iodine number on the same ration without the oil supplement. The expeller, crude, and refined, soybean oil supplements all had an iodine number of approximately 127 and the resulting butter fat from the supplements all had an average iodine

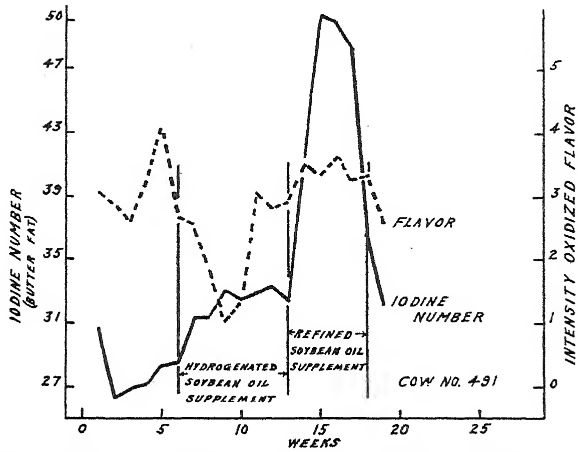

FIG. 4. Effect of fat in the ration on the development of oxidized flavor in milk.

TABLE 2

The relationship of iodine number of oil supplement in the ration to the iodine number of butter fat

Oil supplement	Iodine number of oil supplement	Butter fat			
		Number of samples	Range of iodine numbers		
			Highest	Lowest	Average*
No supplement	....	160	38.1	22.2	28.1
Cocoanut oil	11.2	38	38.9	21.5	24.2
Expeller soybean oil	126.7	12	52.0	36.3	45.6
Crude soybean oil	127.5	12	52.9	35.3	43.7
Refined soybean oil	126.6	38	51.6	39.2	45.7
Hydrogenated soybean oil	17.4	38	35.5	28.2	33.5

* All transition values excluded.

number of approximately 45. These supplements were high in the longer-chain unsaturated fatty acids. In addition to this the expeller oil contained 4.20 per cent phosphatides calculated as lecithin whereas the crude soybean oil contained 1.91 per cent and the refined soybean contained .061 per cent. The hydrogenated soybean oil contained .028 per cent lecithin whereas the cocoanut oil contained .035 per cent lecithin. The phospholipids were determined by the method outlined by Horrall (5) and the lecithin calculated from the phosphorus. From these results so far as could be determined by inspection the percentage of lecithin in the oil supplement had little or no effect on the susceptibility of the milk to metal-induced oxidized flavor. The

TABLE 3

Variations in iodine-number of milk fat and in metal-induced oxidized flavor of milk from control cows on a low-fat ration during periods corresponding to that of the oil-fed group

Cow number	374		385		441	
Corresponding period	Iodine number	Flavor*	Iodine number	Flavor*	Iodine number	Flavor*
			27.66	1.33	27.59	1.84
			28.66	1.84	26.41	1.17
	29.27	3.66	27.94	2.66	27.56	1.84
	31.63	3.00	27.07	2.66	28.30	1.84
	27.24	1.50	27.25	1.84	25.27	2.66
	24.34	0.66	28.51	2.84	24.84	1.84
Preliminary	22.96	0.66	29.24	2.00	26.69	1.33
	24.75	0.84	28.63	3.00	24.88	1.50
	27.24	1.50	27.17	1.50	26.58	2.66
	20.51	1.00	29.86	2.33	25.57	2.66
	19.71	1.00	28.38	2.00	24.47	2.33
	18.99	0.33	29.00	2.00	25.41	2.84
	20.22	1.50	29.42	2.66	25.55	2.00
Average	24.26	1.42	28.37	2.20	26.09	2.04
	21.50	0.17	29.76	2.00	26.77	2.00
	21.51	0.00	29.41	2.50	25.04	1.33
	21.43	0.33	29.00	1.84	27.02	3.33
	29.03	0.50	29.72	3.33	28.70	3.17
	27.38	0.66	27.98	0.66	27.25	2.00
	25.45	1.00	30.90	1.50	27.21	2.50
Oil feeding	25.26	0.33	31.01	2.00	27.02	2.66
	22.64	1.33	29.23	1.66	26.47	2.33
	22.20	1.00	29.38	2.33	25.98	2.33
	28.23	0.84	30.27	3.50	26.03	2.33
	24.01	0.33	30.63	2.50	27.01	4.84
	23.58	0.17	30.74	2.84	25.10	3.17
	24.16	0.33	30.99	2.00	26.34	2.50
Average	24.34	0.54	29.92	2.20	26.61	2.65
	23.31	1.00	31.12	4.33	28.37	3.84
	22.94	1.00	31.13	2.00	28.18	3.66
	22.76	0.50	30.47	1.50	25.76	2.66
	22.71	0.33	30.45	2.66	26.24	2.33
Oil reversal	23.77	0.00	31.59	1.17	26.16	0.66
	22.93	1.17	31.22	2.66	41.25	1.00
	21.96	0.33	30.17	1.84	35.07	0.17
	22.05	1.84	30.87	2.33	27.89	2.33
Average	22.80	0.77	30.88	2.31	29.87	2.08
	23.02	1.17	33.28	0.50	27.49	1.00
	23.06	0.66	31.10	1.50	27.92	0.66
	21.82	0.17	30.66	1.00	27.53	2.84
Readjustment	32.16	0.17	31.66	1.00	29.06	3.00
	22.38	0.00	32.16	0.33	28.22	3.00
	23.25	0.50	31.80	1.33	28.97	4.00
	24.12	0.33	31.53	0.33	30.35	2.66
	22.84	0.00	31.38	1.66	29.71	3.00
	26.26	0.00	35.62	0.50	28.99	1.33
Average	24.32	0.33	32.13	0.91	28.69	2.39

* See Table 1 footnote.

hydrogenated soybean oil supplement which is comparable with the refined soybean oil except that the iodine number had been reduced to 17.4 by hydrogenation, resulted in butter fat with an average iodine number of 33.5, compared to 28.1 for butter fat produced without an oil supplement. This indicates that the cow can desaturate the fat during the metabolic process.

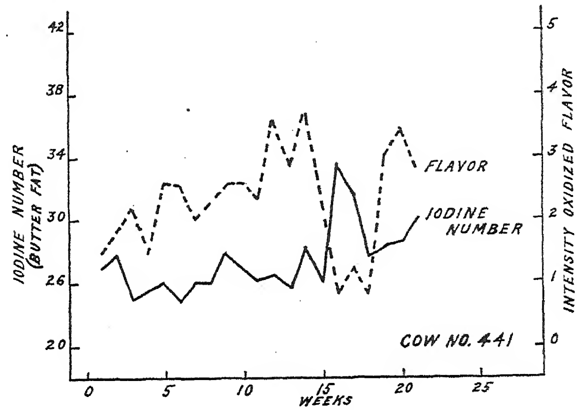

FIG. 5. Variations in iodine number of butter fat and in metal-induced oxidized flavor of milk from a control ration.

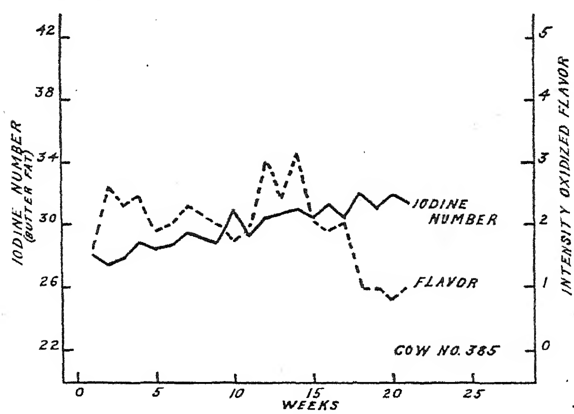

FIG. 6. Variations in iodine number of butter fat and in metal-induced oxidized flavor of milk from a control ration.

The relationship between iodine number fluctuation and the intensity of metal-induced oxidized flavor on a normal ration is shown in figures 5 and 6 and in table 3. An inspection of these data reveals that small changes in iodine number of the butter fat has little influence on the intensity of metal-induced oxidized flavor in milk. Apparently, it is of minor importance when compared to other factors.

DISCUSSION OF RESULTS

The results obtained in this experiment indicate that there is a direct relationship betwen the iodine number of the fat in the ration and the intensity of metal-induced oxidized flavor developed in the milk. As the iodine number of the butter fat increases the intensity of the oxidized flavor increases. This does not follow in the case of feeding hydrogenated soybean oil. In this case the cow apparently desaturates the fat in the ration and the iodine number of the butter fat increases over that of a normal ration. The hydrogenated soybean oil was very laxative while the other oils did not display this tendency. The results obtained from the control cows on a low fat ration indicate that there is little correlation between the iodine number of the butter fat and the intensity of metal-induced oxidized flavor developed in the milk. Apparently other factors are much more important and their fluctuation obscures any relationship which might exist.

The results obtained in this experiment are not in agreement with those of Corbett and Tracy (1), but in their work the trials were conducted for a period of 10 days, followed by a 10-day readjustment period prior to a 10-day reversal in type of oil. During a major part of that time the animals were not receiving as large a supplement as in the present experiments. In our experiments the cows were maintained on the oil for a period of seven weeks followed by a five-week reversal in type of oil. Under these feeding conditions of one pound of oil per cow per day it required approximately three to four weeks for the iodine number of the butter fat to reach a maximum change. This is a somewhat longer period than has been reported previously.

Other interesting conditions are the range in fluctuations of iodine number of butter fat due to type of oil consumed in the ration, and the tendency on the part of the cow to produce butter fat of an intermediate iodine number rather than to follow the extremes of the oils found in the ration.

SUMMARY

1. The feeding of 1 pound per day of cocoanut oil in the daily ration of a dairy cow decreased slightly the iodine number of the resulting butter fat and reduced slightly the intensity of the oxidized flavor developed by copper.

2. The feeding of 1 pound per day of either expeller, crude, or refined soybean oil in the daily ration of a dairy cow increased greatly the iodine number of the resulting butter fat and increased the susceptibility of the milk to metal-induced oxidized flavor.

3. The difference in the lecithin content of the different types of soybean oils produced no noticeable change on the development of oxidized flavor.

4. The feeding of 1 pound per day of hydrogenated soybean oil in the daily ration of the dairy cow increased slightly the iodine number of the

resulting butter fat. This is believed to be due to the desaturation of this fat by the cow.

5. In rations low in fat the change in iodine number of the butter fat does not appear to be correlated with the intensity of oxidized flavor developed. Apparently other factors are of more importance.

6. In these experiments the range in iodine number of butter fat has been from 23 as an average for the cocoanut oil supplement to 48 for refined and expeller soybean oil.

7. Under the conditions of these experiments, approximately three weeks were required before the iodine number of the butter fat had reached the period of maximum change.

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THE FREEZING AND THAWING OF MILK HOMOGENIZED AT VARIOUS PRESSURES*

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Although many observations and studies have been made on the effects of partial freezing on the distribution of fat and solids-not-fat of milk, few such investigations have been made on the freezing of homogenized milk.

Very soon after Vieth (24) in 1886 reported noting different qualities between frozen and unfrozen portions of milk and that the fluid part of partially frozen milk was richer in fat than the frozen part, Henzold (12) conducted several experiments on partial freezing of milk, showing that the fat content of the milk-ice was dependent in large part upon the creaming of the milk. If creaming occurred prior to freezing the milk ice was higher in fat than the fluid part and vice versa. Other workers, Richmond (19), Farrington (9), Bischoff (5), Mai (14), Utz (23), Winter (25), and Koenig (13) presented further evidence showing that the relative richness of the frozen and unfrozen portions of milk was dependent upon the extent of freezing and creaming. Later, Baldwin and Combs (1) showed that the freezing of the cream resulting from the fat rising before freezing began apparently accounted for the greater percentage of fat in the frozen portion. Baldwin and Doan (3) found that when the creaming of milk was destroyed by heating or by homogenization the fat concentration of the unfrozen portion increased as freezing progressed while that of the frozen portion was decreased at first but finally approached the fat percentage of the original milk as freezing approached 100 per cent.

When the milk was frozen solid the percentage distribution of constituents was found by Bordas and Raczkowski (6), Besana (4) and Fascetti (10) to vary widely depending upon the portions analyzed and the conditions of freezing. Doan and Baldwin (8) observed that if the fat content exceeded 25 per cent the product froze homogeneously when frozen unagitated, and that homogenization caused homogeneous freezing at a lower fat content.

Not only has the composition of milk been found to be affected by the freezing, but by the thawing as well. Pade (16) and Winter (25) noted a great variation in the composition of various portions of fractionally thawed frozen milk, that first obtained on thawing being quite rich in total solids.

Freezing of milk has often been considered as affecting the creaming of milk. Reid (18) observed that freezing greatly intensified the clumping of the fat globules. However, he found that the depth of the cream layer might be increased or decreased according to the conditions of freezing.

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Baldwin and Combs (2) concluded that the manner of thawing played the major role when decreases in the efficiency of creaming of partially frozen and thawed milk occurred.

A flaky appearance is commonly observed in thawed frozen unhomogenized milk. However, precipitation of solids of milk as a result of freezing has not been so generally noted. While Reid (17) observed that the milk serum after freezing and thawing was bluish and had a watery appearance he made no mention of the presence of a "ring of sediment or precipitate of a gelatinous-like character" as observed and analyzed by Munkwitz and coworkers (15). The Connecticut Station (7) reported that the precipitation of casein was not influenced by homogenization when the milk was held at $-14°$ F., but when stored at $+10°$ F. a pressure of 4000 pounds per square inch caused a precipitation of casein approximately twice as rapid as that homogenized at 2000 pounds, which had a slight effect when held under similar conditions.

Gooren (11) concluded from data of two trials that homogenization of milk slightly lowered its freezing point.

Inasmuch as homogenized milk sales are increasing and that few studies on freezing and thawing of milk involved the homogenized product a study of some of their effects on certain properties of homogenized milk seemed desirable.

EXPERIMENTAL

Mixed milk, averaging 3.8 per cent fat, pasteurized in a 200-gallon vat at 143° F. and homogenized at pasteurization temperature, using a 200-gallon per-hour viscolizer, was used throughout the study. The fat content of the various portions was determined by the Babcock method, the total solids by the Mojonnier method, and the solids-not-fat calculated from these two values. The titratable acidity was determined according to the Mann's acid test. Freezing, either partial or solid, was brought about at 0° F. and thawing, either by exposure to air at room temperature or to a water bath at 90–100° F. The samples were set for creaming in a room at 40° F. The various layers of milk in the creaming cylinders were obtained by a water suction pump using a trap within the suction line. Samples of drainage from thawing milk were secured by exposing to room temperature the stripped quart "Sealrights," placed on a triangle over a funnel.

RESULTS

Rate of freezing. Mixed milk homogenized at 0, 500, 1500, 2500 and 3500 pounds pressure and bottled was tempered at 40° F. for 24 hours and then exposed to 0° F. for 3 hours. At the expiration of this period a one-half inch hole was drilled in the frozen plug, through which the unfrozen portion was poured. The drillings were carefully saved and returned to the frozen portion. The percentage frozen was calculated from the weights of

the unfrozen and of the original milk. The average percentages of five series frozen has previously been reported (22) but is shown graphically in figure 1 as a background for the analytical data being presented in this paper. A slightly higher percentage of the milk homogenized at 2500 and

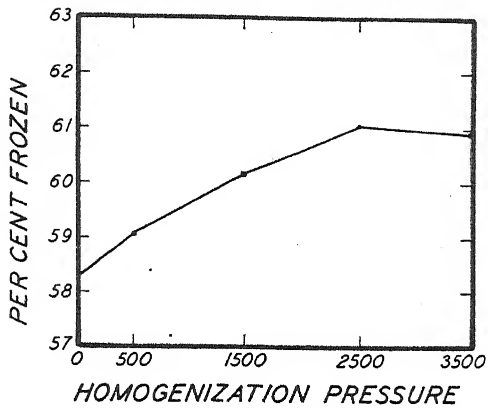

FIG. 1. Per cent of milk frozen at the end of three hours exposure to 0° F. when homogenized at various pressures.

3500 pounds froze during the three-hour exposure than that homogenized at 500 or 1500 pounds or that unhomogenized, the latter showing the least freezing. Despite evidence (11) that homogenization slightly lowers the freezing point of milk, these slight increases in the rates of freezing of homogenized over the unhomogenized milk must be associated chiefly with the different insulation values of the milk ice, the composition of which is influenced largely by the migration of the fat globule.

Analysis of solid and liquid portions of partially frozen milk. The series of milk homogenized at various pressures, previously discussed under rate of freezing, were tested for fat and total solids. The data obtained are illustrated in figure 2. The frozen portion of the unhomogenized milk had a higher fat content than the liquid portion. However, the reverse was true when the milk was homogenized. Despite the higher fat content in the frozen portion of the unhomogenized milk, the total solid content was lower than in the unfrozen portion. The lower total solid content in the frozen portion was noted also in the homogenized samples. However, the difference in the total solid content between the frozen and unfrozen portions of the unhomogenized milk was markedly less than that noted in the homogenized samples. In these trials in which the milk was frozen approximately 60 per cent, the unfrozen portion contained approximately 2.50 to 2.75 per cent more solids-not-fat than did the frozen portion, regardless of homogenization.

Five separate lots of milk heated to inhibit creaming exhibited the same trend in distribution of fat and solids as did the homogenized milk upon

similar partial freezing. These data confirm results of other investigations showing that the inhibition of creaming and not the decrease in the size of the fat globule was largely responsible for the lower fat content in the frozen portion than in the unfrozen portions of partially frozen homogenized milk.

The difference in the fat content of the frozen and unfrozen portions of the high-heat-treated milk was remarkably close to that noted when the milk was homogenized at low pressure on which no creaming was observed.

It should be pointed out again, however, that efficiency of the homogenization, beyond the pressure necessary to inhibit creaming, seemed to exert a slight influence on the rate of freezing, and, in this connection, analyses show a slightly greater difference between the fat content of the frozen and

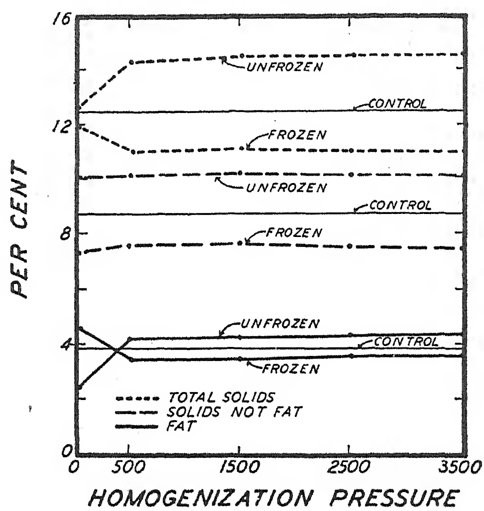

FIG. 2. Per cent fat, solids-not-fat and total solids in the frozen and in the unfrozen portions of bottled milk after three hours at 0° F. when homogenized at various pressures.

unfrozen portions as the pressure of homogenization was increased. However, this slight difference in composition attributed to pressure might be due to the slightly greater percentage frozen as a result of the pressure of homogenization.

Distribution of titratable acidity in partially frozen milk. The titratable acidity was found to be approximately 50 per cent higher in the liquid portion than in the solid portion of the partially frozen milk and showed no variation regardless of the pressure of homogenization. This distribution of titratable acidity seemed to follow quite closely the distribution of the solids-not-fat in the frozen and in the unfrozen portions.

The homogeneity of milk homogenized, frozen, and thawed under different conditions. Five series of mixed milk were pasteurized, divided into five lots and homogenized at 0, 500, 1500, 2500, and 3500 pounds respectively. Three 100-ml. cylinders of each lot were set at 40° F. for creaming.

After 24 hours two of the cylinders of milk were frozen solid at 0° F. for at least 12 hours. One cylinder was then set in a water bath at 90 to 100° F. while the other cylinder was exposed to the air at 70–75° F. When all traces of ice had disappeared the cylinders were again set at 40° F. for 24 hours for further creaming. The upper and the lower 50 ml. of milk were then drawn into separate half-pint milk bottles using a water suction pump.

The homogeneity of the milk was found to have varied greatly as a result of freezing and the rate of thawing and to a much lesser extent to the pressure of homogenization (figs. 3, 4, and 5).

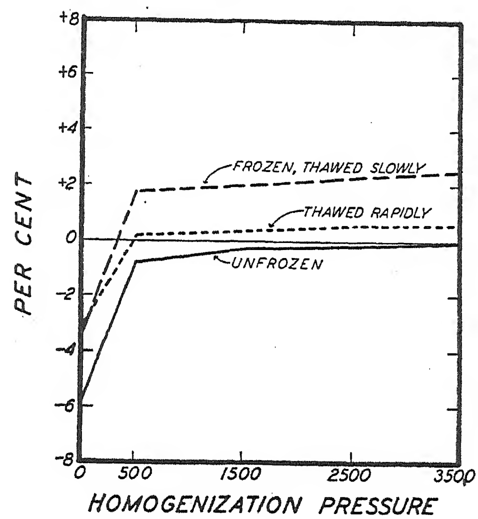

FIG. 3. The fat content of the lower half of creaming cylinders of milk with respect to that of the upper half when the milk was homogenized at various pressures, was frozen and was thawed under different conditions.

When homogenized milk remained unfrozen and was creamed for 48 hours there was a tendency for the fat to rise, the upper 50 ml. showing upon analysis a slightly higher fat content as well as a very slightly higher total solid and solids-not-fat content than the lower 50 ml. However, when the homogenized milk was frozen and thawed, slowly or rapidly, settling rather than creaming was encountered, as the lower 50-ml. portions always contained a markedly higher fat and total solids content. Especially was the settling out of the fat and total solids pronounced when the frozen milk was allowed to thaw slowly. The difference in analysis between the upper and lower layers seemed to be greater in case of the solids-not-fat than with the fat. Apparently the destabilized casein resulting from freezing and thawing although not showing flakiness in the homogenized milk, as commonly observed in frozen unhomogenized milk, swept down the fat as the heavier solids settled or else the fat globules, small as a result of homogeni-

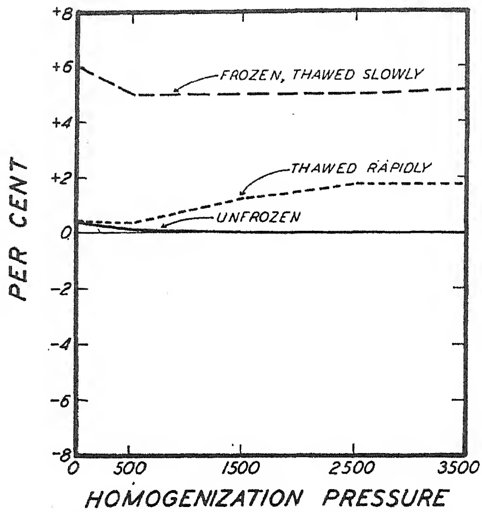

FIG. 4. The solids-not-fat content of the lower half of creaming cylinders of milk with respect to that of the upper half when the milk was homogenized at various pressures, was frozen and was thawed under different conditions.

zation, were weighted down through adsorbed destabilized casein. As previously reported (22), frozen homogenized milk when thawed under certain conditions presents a watery appearance in the surface layers. Analysis of the upper 15 per cent, middle 70 per cent, and lower 15 per cent of creaming cylinders of milk frozen and thawed as previously described furnish further

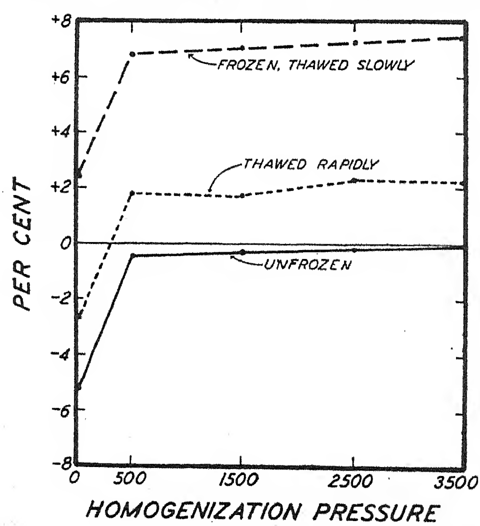

FIG. 5. The total solids content of the lower half of creaming cylinders of milk with respect to that of the upper half when the milk was homogenized at various pressures, was frozen and was thawed under different conditions.

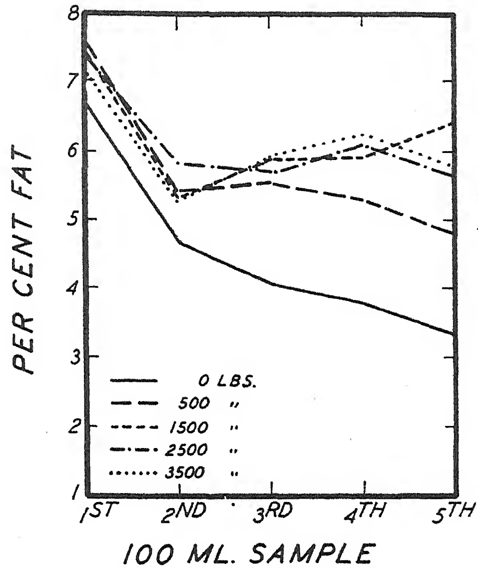

FIG. 6. The fat content of consecutive 100-ml. samples of drainage from quart "Sealrights" of frozen milk exposed to 70° F. when homogenized at various pressures.

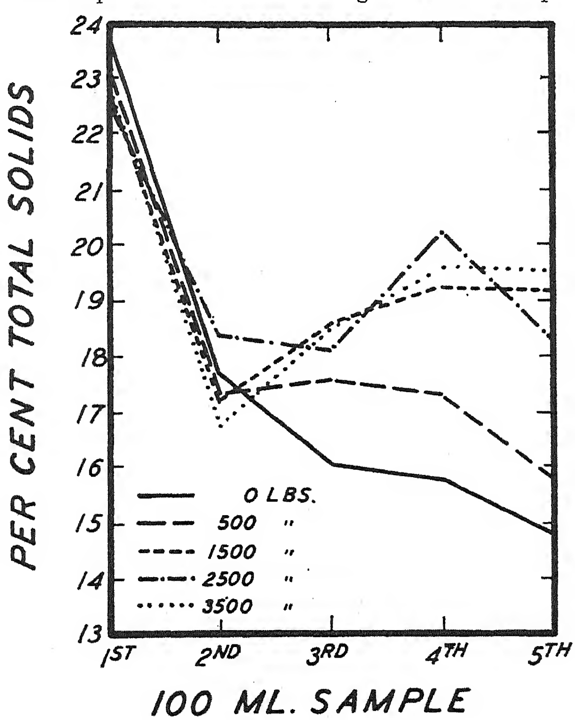

FIG. 7. The total solid content of consecutive 100-ml. samples of drainage from quart "Sealrights" of frozen milk exposed to 70° F. when homogenized at various pressures.

evidence of the settling of fat and total solids of homogenized milk under certain conditions and may serve to explain in part, the often spasmodic occurence of sediment in homogenized milk and its analysis (20, 21). When the milk was homogenized, for example, at 1500 pounds pressure, and was frozen and thawed slowly, the fat, solids-not-fat and total solids of the upper 15 per cent were found to be as low as 1.7, 3.09, 4.69 per cent, respectively, as contrasted to 7.7, 16.90, and 26.60 per cent, respectively, in the lower 15 per cent of the same samples. The titratable acidity in the upper and lower portions of the above samples was 0.04 and 0.35 per cent, respectively.

When the milk was heated sufficiently high to inhibit creaming and frozen and thawed the same as the homogenized milk, the fat tended to rise, forming a cream layer rather than settling as was the case in the homogenized milk.

The upper and lower halves of 100-ml. creaming cylinders of skim milk were studied also. Homogenization seemed to have little influence on the composition of the upper and lower layers but the freezing and rate of thawing had a marked effect as was noted in the whole milk.

The titratable acidity of upper and lower halves of creaming cylinders of milk frozen and thawed under different conditions. The per cents titratable acidity of the upper and lower portions of 100-ml. creaming cylinders of unfrozen milk were found to be practically identical regardless of the pressure of homogenization. However, when the milk was frozen and thawed the per cent acidity was always markedly higher in the lower layers than in the upper layers, the slower the thawing apparently the greater the difference in titratable acidity between the respective portions. Little consistent influence resulting from the pressure of homogenization was noted.

Composition of drainage from frozen milk upon thawing. Quart "Sealright" containers of milk homogenized at 0, 500, 1500, 2500 and 3500 pounds pressure were frozen solid at 0° F. for 48 hours, were stripped of the paper container, and were exposed at room temperature for complete melting. The frozen cylinders of milk were supported on a triangle above a large funnel conducting the drip into a 100-ml. cylinder. Each of the first five 100-ml. portions from each sample was caught separately, and analyzed for fat, total solids and titratable acidity. The data obtained are shown in figures 6 and 7. The data show that the fat drainage was somewhat slower in the unhomogenized than in the homogenized milk, the successive samples of drainage testing less in fat than the corresponding samples from the homogenized milk. Furthermore, the remaining portion after drainage of 500 ml. was higher in fat in the unhomogenized, being 1.90 per cent, than in the homogenized samples, which at 3500 pounds pressure was 0.80 per cent. Pressure of homogenization, except possibly 3500 pounds, in which sample less fat remained after removal of 500 ml. of drainage, seemed to have little consistent influence on the rate of fat drainage.

The drainage of total solids was found to be slightly faster in the first 100-ml. samples of milk processed at 0 and 500 pounds pressure than at 1500, 2500 or 3500 pounds pressure (fig. 7). However, this faster drainage of total solids in the unhomogenized milk was of short duration for in the third, fourth and fifth 100-ml. samples the total solid content became markedly less than in the respective samples of homogenized milk. Despite the higher total solids content of the first sample of drainage of unhomogenized milk, the remainder of the quart, after collecting five 100-ml. samples, contained more total solids than did those from the corresponding samples of homogenized milk.

The distribution of solids-not-fat and titratable acidity was quite similar to that of the fat and total solids. Similar studies were made on two series of skim milk. The data obtained on the respective samples were comparable to those found in the study of whole milk, namely, a slightly faster drainage of the total solids as a result of homogenization.

SUMMARY

The analyses of various portions of milk homogenized at different pressures and frozen partially or wholly have been made.

A slightly greater percentage of homogenized than unhomogenized milk froze in a given period at 0° F.

When creaming was inhibited by heating or by homogenization the unfrozen portion was relatively richer in fat and solids-not-fat than the frozen portion. When creaming occurred, as in the unhomogenized milk, the frozen portion was higher in fat but lower in solids-not-fat than the unfrozen portion.

The titratable acidity of the unfrozen portion was higher than the frozen portion, regardless of creaming. No evidence of creaming was noted in frozen homogenized milk but rather a pronounced settling of the fat which was influenced by the rate of thawing.

Frozen homogenized milk upon thawing exhibited no flakiness which was commonly observed when unhomogenized milk was frozen and thawed, but did exhibit a watery appearance at the surface layers which was more pronounced when the frozen milk was thawed slowly.

Marked settling of the fat and solids-not-fat of milk was noted when homogenized milk was frozen and then thawed. The rate of thawing had a pronounced influence upon the extent of settling of the fat and solids-not-fat; the lower 15 per cent of creaming cylinders of slowly thawed frozen homogenized milk contained as high as 7.7 per cent fat and 24.60 per cent total solids as contrasted to 2.0 and 5.50 per cent, respectively, of the upper 15 per cent layer. The titratable acidity of the upper and lower 15 per cent portions ranged from 0.04 to 0.35 per cent. Similar trends were observed upon freezing and thawing of skimmed milk. When milk was heated to

inhibit creaming was frozen and thawed, the fat tended to rise to form a cream layer, a contrast to the movement of fat in the thawed frozen homogenized milk.

The drainage of solids from homogenized milk was slightly faster after the first 100 ml. than that of the unhomogenized milk.

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SOME FACTORS AFFECTING THE BODY OF MARKET CREAM*

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The body of market cream refers to its appearance and is the commercial terminology employed to designate resistance to flow or deformation. This somewhat intangible property is a visual indication of quality to many consumers. Cream that flows slowly is regarded as having a high fat content and considered suitable for most purposes if the other inherent quality factors are present.

Dealers frequently encounter situations when their product shows a thin consistency and is frequently criticized as being deficient in fat, even though it meets the class requirements. Conversely, consumers sometimes complain of cream being so viscous that it will not readily pour from the bottle. Because of this consumer reaction that may not always be justified, the body of market cream has assumed commercial prominence and the dealer is continually seeking methods of processing that will aid in producing cream of uniform and acceptable body.

The literature on this subject was read by the authors. Detailed citations are omitted. Sommer (4) has contributed an excellent review of the fundamental factors involved in the viscosity of cream.

PROCEDURE

An approach to this problem was made through a study of creams subjected to various treatments and involving seasonal factors. The cream was obtained from mixed Ayrshire, Guernsey, Holstein and Jersey milk at the University Farm. The milk received at 40° F. was warmed to 80° F. within 15–20 minutes and then centrifugally separated. The cream was standardized with its own skimmilk.

When washed (3), the fresh cream was diluted with four volumes of water at 100°–110° F. and reseparated in a centrifugal machine, this process being repeated one to five times on different lots. The washed cream was standardized with water and the normal cream with its own skimmilk to the desired fat content. The creams were pasteurized for 20 minutes at 145° F. and cooled to 40° F., being gently agitated during heating and cooling, but not during holding.

Samples for viscosity determinations were taken in half pint bottles after the cream was cooled and held at 40° F. ± 2°. The measurements were made after 4 and 24 hours with the Borden Body Flow Meter designed

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by Nair and Mook (2) with an outlet of approximately 3.75 mm., each sample being poured into a second cold half-pint bottle, then into the cold test bottle and flow time measured in a laboratory temperature of 70–75° F. The increment in temperature due to handling and contact with warm air did not exceed 2° F. The viscosimeter was held at 40° F. prior to using. The average values for triplicate determinations of creams with a viscosity of less than 100 seconds rarely varied more than ± 1 second and for the more viscous creams did not usually vary more than ± 3 seconds from the mean.

The Borden Body Flow Meter has limitations not within the scope of this paper. Suffice it to state that comparisons between washed and normal cream are not on a comparable basis because of differences in specific gravity.

The experimental creams were observed for clustering of the fat globules with an oil immersion objective and a paraboloid condenser. They were diluted 25 times with their own skimmilk, a more satisfactory diluent than glycerine and water. All microscopic examinations were made at 40° F. ± 3°.

RESULTS

The viscosity of normal and of washed creams. Table 1 shows viscosity determinations on normal and washed creams, the latter being washed five

TABLE 1

The effect of washing on the viscosity of cream

Month	Trial No.	Fat	Age	Viscosity in seconds			
				Normal cream		Washed cream	
				Raw	Pasteurized	Raw	Pasteurized
1937		*per cent*	*hours*				
November	1	35	4	*	79.9	30.6	26.6
			24	*	132.0	34.8	28.0
November	2	35	4	*	79.2	33.3	23.1
			24	*	133.6	33.4	24.0
December	3	35	4	*	53.8	38.0	30.6
			24	*	94.1	43.0	38.0
December	4	35	4	201.6	72.5	37.5	31.9
			24	316.7	114.3	42.9	37.7
1938							
January	5	35	4	265.5	35.2	23.2	20.5
			24	341.2	40.4	24.5	21.5
February	6	35	4	*	44.8	45.0	35.7
			24	*	50.7	49.9	45.0
March	7	30	4	268.8	39.2	24.2	22.4
			24	*	44.4	26.4	23.4
April	8	30	4	128.4	40.2	24.9	22.9
			24	236.0	44.8	27.2	25.2
June	9	30	4	94.5	39.7	27.5	23.7
			24	144.5	42.2	29.4	26.2

* Too viscous to measure.

times. The data reveal in some cases that the raw cream was too viscous to flow. This was true in the trials conducted while the cows were on winter feed and such extremely high viscosity may be due to the physico-chemical state of the butterfat. It is a well known fact that fats of the lower melting points increase when the cows are pastured.

The viscosities of the pasteurized creams increased with age in the trials conducted during November and December, while during January to June the increases were small, in fact during this period the viscosity of the pasteurized creams did not change greatly with season. Washing cream greatly reduced the viscosity that was only slightly affected by pasteurization and age. This indicates that the plasma constituents are involved in viscosity changes with pasteurization and with age.

Effect of temperature treatment on the viscosity of pasteurized normal and washed cream. Table 2 shows viscosity values on temperature treated,

TABLE 2

Effect of temperature treatment on the viscosity of pasteurized normal and washed cream containing 30 per cent fat

Month	Trial No.	Age	Viscosity in seconds			
			Normal		Washed	
			Control	Heat treated	Control	Heat treated
1938		*hours*				
June 2	1	4	39.7	68.4	23.7	28.0
		24	42.4	108.2	26.2	36.8
June 9	2	4	37.0	72.1	24.1	28.9
		24	44.2	170.5	27.7	41.3
June 13	3	4	34.0	60.7	30.3	39.7
		24	40.0	152.6	35.0	68.3

normal and washed creams from milk produced while the cows were on pasture during June. The pasteurized creams were aged for 12 hours at 40° F. and subjected to the method of Hening and Dahlberg (1) for increasing viscosity. It is evident that the temperature treatment increased the viscosity; however, the increases for washed cream are not great. Results of the eight winter trials conducted during November to April are not shown because most of the temperature treated creams would not flow through the viscosimeter. These creams resembled a gel, and on standing at 40° F. in test tubes showed syneresis. This condition existed until the cows were pastured when the reaction to temperature treatment became less intense so that viscosity could be measured.

Effect of a modified temperature treatment. These experiments were conducted during the late fall and winter when the reaction to heat treatment was intense. The creams were pasteurized and quickly cooled to 82° F. Portions were held for varying lengths of time, followed by slow cooling in 20–30 minutes to 40° F. Control portions were cooled to 40° F.

Table 3 indicates that this processing reduced viscosity below that of the control. A slight tendency toward higher viscosity is indicated for the cream held at 82° F. for 20 minutes as compared to the other periods.

TABLE 3

Effect of a modified temperature treatment on the viscosity of 30 per cent cream

Month	Trial No.	Age	Viscosity in seconds				
			Pasteurized at 145° F. for 20 minutes				
			Control	Cooled to 82° F. and held for			
				10 min.	20 min.	30 min.	40 min.
1937		*hours*					
November	1	4	79.2	67.7	68.6	69.2	63.2
		24	133.8	85.2	89.0	87.4	73.9
November	2	4	65.8	58.7	59.9	60.2	59.6
		24	118.8	75.1	83.1	80.1	75.4
1938							
January	3	4	78.2	70.2	73.2	72.9	72.2
		24	126.5	82.4	84.4	83.0	80.2

However, the differences are of little significance since they were apparent only when measured by the viscosimeter. The controls showed higher viscosities in all cases. All the creams, except the controls, contained some free fat indicating destabilization of the emulsion.

Effect of repeated pasteurization and temperature treatment. These trials were made during the w'nter when the reaction to temperature treatment was intense. Normal cream and a portion of the same cream washed five times, pasteurized and promptly cooled to 40° F. was used for each trial. After aging for three hours, the creams were temperature treated, cooled and held at 40° F. After 24 hours they would not flow through the viscosimeter. They were again pasteurized and heat-treated, followed by another processing. In all, these creams were pasteurized and temperature treated three times. The results set forth in table 4 show that the first treatment increased the viscosity which was subsequently lowered by pasteurization. A second treatment reacted and the viscosity was increased only to be again lowered by a third pasteurization. This indicates that the substance or substances responding to heat treatment are not affected greatly by pasteurization.

Seasonal variation in the viscosity of pasteurized and of temperature treated cream. These trials were conducted during April 21 to July 19 and included winter and summer feeding. The cows were pastured on May 9. The evening milk was held over night at 40° F. and separated the following morning at 80° F. The cream for each trial was standardized, pasteurized, cooled rapidly to 40° F. with iced water and samples taken for viscosity determinations. A portion of the same cream was held at 40° F. for approximately 12 hours and was temperature treated.

TABLE 4

The effect of repeated pasteurization and temperature treatment on the viscosity of cream containing 30 per cent fat

Treatment	Age	Viscosity in seconds					
		Normal			Washed		
		Trial number					
		1	2	3	1	2	3
	hours						
First pasteurization	24	72.2	79.9	70.3	23.1	26.3	29.2
Heat treated	24	*	*	*	*	*	*
Second pasteurization	48	75.4	76.8	68.7	22.4	25.6	27.4
Heat treated	48	*	*	*	*	*	*
Third pasteurization	72	70.2	72.0	66.8	21.2	24.2	26.6
Heat treated	72	*	*	*	*	*	*

* Too viscous to measure.

The results set forth in table 5 show but little variation in viscosity of the pasteurized cream after aging for 24 hours. This was unexpected as it had been experienced that most complaints of poor cream body were associated with the transition from winter to summer feed. On the other hand

TABLE 5

Seasonal variations in the viscosity of pasteurized and of temperature treated cream containing 30 per cent fat

Month	Trial No.	Viscosity in seconds			
		Pasteurized			
		Normal		Heat treated	
		4 hrs.	24 hrs.	4 hrs.	24 hrs.
1938					
April 21	1	43.1	50.2	*	*
25	2	36.0	43.1	*	*
29	3	42.6	49.8	*	*
May 3	4	45.7	60.8	*	*
5	5	41.5	46.1	*	*
9	6	41.1	45.8	*	*
12	7	40.9	46.3	206.4	*
16	8	41.3	43.7	239.9	*
19	9	39.8	43.6	157.3	311.0
23	10	40.2	44.1	97.2	161.2
26	11	49.1	57.0	88.4	161.9
30	12	43.3	46.9	86.4	158.1
June 2	13	35.2	43.1	95.5	140.1
9	14	42.5	47.1	91.1	142.3
16	15	43.0	47.6	90.8	157.0
23	16	39.9	49.4	93.8	177.5
30	17	47.2	54.6	91.0	174.9
July 7	18	42.7	50.6	*	*
12	19	40.3	49.4	*	*
19	20	42.4	49.2	*	*

* Too viscous to measure.

significant changes were evident in viscosity of the heat treated creams with season when the cows were pastured on May 9. This condition prevailed for about six weeks when the creams again increased in viscosity by heat treatment so that flow-time could not be measured. No consistent relationship was evident between fat globule clumping and viscosity. This experiment was repeated through the same period in 1939 with similar results.

The stability of cream body. The purpose of the following trials was to determine the resistance of the body of market cream to handling. Cream was transferred from one halfpint bottle to another and this constituted one transfer in table 6. Successive transfers were made in denominations of ten and the viscosity measured. The trials were performed in a refrigerator at 45° F. ± 3° F. with forced circulation of air. It required approximately 1½ hours to complete the tests on each sample of pasteurized or heated cream that was previously aged for 24 hours. The increase in temperature due to handling the apparatus and the cream was 4–5° F.

The trials with pasteurized cream were made during January while those with pasteurized temperature treated cream were conducted during June. The results in table 6 indicate that cream has a temporary structure

TABLE 6

The stability of cream body

No. of transfers	Viscosity in seconds					
	Pasteurized 35 per cent			Pasteurized heat treated 30 per cent		
	Trial number					
	1	2	3	1	2	3
1938						
1	162.0	130.8	152.0	249.8	270.2	135.2
10	158.2	127.4	139.4	178.0	168.4	115.0
20	154.8	120.2	126.8	141.0	148.6	104.8
30	142.2	116.6	120.0	116.4	138.4	97.4
40	131.0	109.0	114.0	95.2	120.0	90.2
50	124.0	102.4	107.8	82.8	114.0	84.6
60	119.2	98.0	101.0	75.0	108.2	76.2
70	112.0	93.4	98.2	71.4	104.4	70.0
80	104.0	90.2	94.8	66.2	100.0	68.4
90	99.2	87.8	90.0	62.0	94.0	66.2
100	95.4	83.0	85.6	58.2	89.8	62.8
110	88.6	79.8	81.4	56.2	81.4	54.8
120	85.2	77.0	79.2	54.2	75.6	53.8
130	82.4	76.2	78.8	53.8	70.2	52.4
140	83.2	76.4	78.4	54.0	68.4	52.6
150	82.0	76.2	78.6	54.0	68.6	52.4
160	82.4	76.2	78.4	53.8	68.4	52.4
170	82.2	76.2	78.4	53.8	68.4	52.4

that may be reduced to a constant value under standard conditions. The six samples of cream showed slight clumping with no difference in degree

of clumping of the fat globules at the beginning and at the end of each trial.

DISCUSSION

The removal of most of the plasma constituents greatly reduced the viscosity of washed as compared to normal cream. The viscosity of the former did not vary greatly during the seasons studied. The washed and normal creams responded decidedly to the temperature treatment during the winter while responses during the summer were relatively less. This may be attributed to the predominance of higher melting point fats in the winter with resultant alterations in some physico-chemical properties.

The fact that the washed cream responded to temperature treatment that had not, previously, been greatly affected by successive exposures to pasteurization, indicates some thermal stability for the materials that are activated to increase viscosity. Furthermore, a certain degree of solidification is necessary to obtain maximum effects. It is probable that this condition might exert a greater effect in washed cream because of the reduced viscosity by removal of plasma colloids, thus allowing fat globule clusters to rise more rapidly and pack more solidly.

The body of cream is a temporary structural condition that can be altered by treatment. The formation of this structure in bottled cream has some practical significance in view of the fact that aging at low temperatures increases the viscosity. It is questionable if this structure is the same throughout the entire contents of the bottle. Fat rises and the plasma separates on the bottom, therefore, the fat content is higher in the upper part of the bottle and consequently the cream in this region would have a higher viscosity. Thus, the consumer pouring from a bottle would see the more viscous cream first and form immediate impressions as to the body. This condition would be intensified the longer the cream is held in a bottle or in a larger container at low temperatures. It is inevitable that fat globules rise and strike the viscous surfaces of one another and adhere to form temporary structures that contribute to the apparent viscosity.

From a practical standpoint the viscosity of cream is important insofar as its adaptability to the various uses is affected. There is no proof that extremely viscous cream that pours with difficulty is any more suitable for most manufacturing and household purposes than less viscous cream of the same fat and solids-not-fat content. Granting that viscous cream is produced, the question of handling this product must be considered. The losses that inevitably occur through sticking to equipment and bottles and added refrigeration are of importance.

CONCLUSIONS

1. The washed and the normal creams responded to temperature treat-

ment during the winter, but the responses were less during the summer, especially during the period of transition from winter to summer feed.

2. The viscosity of pasteurized cream aged for 24 hours was not greatly influenced by the transition from winter to summer conditions.

3. The viscosity of cream aged 24 hours and that imparted by temperature treatment is due in part to the formation of a temporary structural condition.

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THE RELATIONSHIP BETWEEN TYPE AND PRODUCTION

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The distinguishing characteristics between various breeds of livestock are principally conformation and color. It is true that all breeds of farm animals possess other characteristics in varying degrees such as rapidity of growth, fattening ability, fecundity, disposition, and in the case of dairy cattle milk yield and butterfat percentage and gait in the case of horses. With dairy cattle, there are also additional characteristics or traits identified with certain breeds, such as size or body weight, rate of maturity, grazing ability, temperature response, persistency of production and color of product.

Without differences in conformation and color, it would be difficult to distinguish one breed of livestock from another of the same species, whether the species be swine, horses or dairy cattle. If our different breeds of livestock are to be maintained, it is essential that standards of type or conformation be continued. Such standards may be flexible, changing with market demands as demonstrated in the swine industry during the past few decades. The value of breed standards of conformation is universally recognized in livestock circles. Without such standards, there can be no such thing as the show ring. Obviously, most of our present standards, especially those related to dairy cattle have been handed down to us. Our ideas of type and beauty in a dairy cow are largely the result of what we have been taught from the cumulative experience of past breeders. Production is of paramount importance in the dairy industry and consequently any standard of type or conformation for a dairy breed must either be definitely associated with producing capacity or at least not inimical to it. Certainly, it must be readily possible to combine both desirable type and high production in the same animal; otherwise, our breed standards of type are wrong.

The Jersey Herd Classification program furnishes perhaps the best yardstick for the measurement of conformation expressed by the score card or scale of points adopted by the American Jersey Cattle Club. The classification program, established in June, 1932, has grown steadily in popularity. Three years ago, a preliminary study was made regarding type and production and the results published in the JOURNAL OF DAIRY SCIENCE. At that time, however, the number of animals available was limited, especially in the case of classified bulls with classified progeny. Since then, a much larger number of animals has been classified and the number is now sufficient to furnish dependable material for study in determining the degree

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of relationship between the producing ability of a cow and her conformation.

Cows and bulls are classified by judges approved by the American Jersey Cattle Club and the cattle are given one of six ratings depending upon their conformation in relation to the scale of points of the Jersey breed. For instance, a cow to be rated "Excellent" must be an animal which in the opinion of the official judge would score at least ninety or more on the official scale of points. A cow to be classified "Very Good" must be an animal which in the opinion of the judge would be entitled to a score of approximately 85 but less than 90 on the official scale of points. The next rating of "Good Plus" is given to animals scoring approximately 80 but less than 85 points. The rating of "Good" is bestowed upon animals which in the opinion of the inspector would be entitled to a score of approximately 75 but less than 80 points. Cows which in the opinion of the judges are entitled to a score of 70 to 75 points are rated "Fair." All animals scoring less than 70 points are classified "Poor."

In applying for Herd Classification, the owner must submit every registered cow he owns that has ever calved and all bulls fifteen months of age or older. No exceptions are permitted. However, it is permissible and the practice is common for breeders to cull their herds (often quite rigidly) before the classifications are made. This explains the decided scarcity of animals that have been classified as "Fair" or "Poor."

Many of the animals that have been classified have also completed official production records either in the Register of Merit or in the Herd Improvement Registry. The records of these cows have been tabulated and converted to a mature yearly equivalent basis, using the American Jersey Cattle Club conversion factors. Table 1 shows the records of all the cows that have been classified to March 31, 1940.

TABLE 1

Production records of all animals classified to March 31, 1940

Classification groups	Total No. of cows classified	Number of cows with R. of M. or H.I.R. records	Per cent of cows with records	Average mature production of each group
Excellent	405	335	82.72	649.59
Very Good	1654	1164	70.37	614.65
Good Plus	2993	1890	63.15	590.28
Good	1936	1058	54.65	553.03
Fair	287	139	48.43	550.29
Poor	15	1	6.67	557.00
Totals	7290	4587	62.92	591.45

The information in this table has been previously published based on a smaller number of records and attention has been called to the relationship between the ratings of the animals classified and the production rec-

ords. In examining this table, it should be observed that a much greater percentage of the higher rating animals have production records than the lower rating animals. This very fact makes an accurate analysis of the data difficult. Nevertheless, the correlation between the production records and the classification records was determined. In making these calculations, two methods were employed. In table 1, there were 139 cows rated "Fair" with production records. These were all used and then an equal number of cows classified "Good" with production records were selected by random sampling. Likewise, 139 cows rated "Good Plus," 139 rated "Very Good" and 139 rated "Excellent" having production records were selected at random. This gave a total of 695 classified cows with production records. In the first calculation, each cow rated "Excellent" was given an arbitrary classification score. It is required that all animals rated "Excellent" be scored in detail by the judge and the average score of the cows that have been rated "Excellent" is 91.5. Thus each cow rated "Excellent" was given this score of 91.5. Each cow classified "Very Good" was asigned a score of 87.5. Each cow classified "Good Plus" was given a score of 82.5, each cow rated "Good" was given a score of 77.5 and "Fair" cows were assigned a score of 72.5. The highest production record of each cow was determined and computed to a mature yearly equivalent basis. The production records were then compared with the classification scores and the correlation coefficient was found to be $+.254 \pm .024$.

Another comparison was made with different scores assigned to the various classification groups. For instance, each cow rated "Excellent" was assigned a score of 6, each cow classified "Very Good" was given a type score of 5, each cow classified "Good Plus" was given a score of 4. Then each cow classified "Good" was assigned an arbitrary score of 3 and each cow classified "Fair" was given a score of 2. There was only one cow classified "Poor"' with a production record and this cow was not used but if "Poor" animals had been used in the analysis, they would have been given a score of 1. Then the production records of the 695 cows were correlated with these arbitrary type scores and the correlation coefficient was found to be $+.262 \pm .024$. This correlation is slightly higher than that reported by Dr. Lush (1) comparing classification ratings on Holstein cows with their production records.

These correlation coefficients are low but they are subject to error for the same reason as the averages given in table 1, because an equal percentage of each classification group has not been tested officially for production. To try to correct this defect, another analysis was made. It was found that of all the herds that have been classified, there were 68 owned by breeders that had done Herd Improvement Registry testing for at least a year prior to the date of classification and then continued the herd on test for at least another year after the classification was made. It was felt that

using the ratings of cows classified in these herds, the percentage of animals with records in each group would be more nearly equal. Consequently, all of the cows classified in these 68 herds were used and it was found that 1980 had completed production records. The production records of this group of cows were determined and the results are shown in table 2.

TABLE 2

Production records of all cows classified in herds doing continuous H.I.R. testing

Classification groups	Total No. of cows classified	Number of cows with official production records	Per cent of cows with records	Average mature 365 day production of each group
Excellent	140	124	88.57	637.27
Very Good	532	445	83.65	590.11
Good Plus	1015	840	82.76	574.37
Good	631	495	78.45	546.19
Fair	106	76	71.70	528.09
Totals	2424	1980	81.68	573.03

Table 2 should be compared with table 1, and it will be noticed that there is a slightly greater spread in production between the classification groups. Next, the cows were ranked in order of production and the results are shown in table 3. In the right hand column of this table, the average classification score for each production group is shown using the assigned arbitrary classification scores as previously explained.

TABLE 3

Average type score of classified cows with production records classified in herds doing continuous H.I.R. testing

Production groups	Excellent	Very Good	Good Plus	Good	Fair	Totals	Average score
900 lbs. and over	4	6	13	1	0	24	85.04
800 to 899 lbs.	7	23	45	13	2	90	83.53
700 to 799 lbs.	31	73	101	57	8	270	83.53
600 to 699 lbs.	34	103	176	101	10	424	83.01
500 to 599 lbs.	28	117	228	125	23	521	82.47
400 to 499 lbs.	15	101	201	125	21	463	82.08
Under 400 lbs.	5	22	76	73	12	188	80.74
Totals	124	445	840	495	76	1980	82.55

There were 76 of the cows rated "Fair" with production records. An equal number was selected at random from each of the other classification groups, giving a total of 380 animals. The correlation was then determined between the production records of this group of 380 animals and their classification ratings, using the arbitrary classification scores of 91.5, 87.5, etc. The correlation coefficient was found to be $+.307 \pm .024$. This correlation is slightly higher than the previous correlations but it is still not high enough to be very significant in indicating much relationship between type

and production. However, even with this group of cows, it was found that a slightly higher percentage of the animals rated "Excellent" have production records than the animals in the lower classification groups. This was probably caused by culling the herds, after the classifications were made. In other words, cows rated "Fair" and "Good" were often disposed of shortly after they were classified and the records were not completed. If an equal percentage of each classification group had been tested, the correlation coefficient would undoubtedly be higher.

The possibility of any ten animals picked at random in any classification group excelling any ten others selected at random from a different classification group was of interest and such selections were made six times. In making these selections, ten animals were picked at random from each classification group and compared with ten animals picked at random from each of the other classification groups. The results are shown in table 4, and indicate that selecting ten cows from any classification group will not positively insure higher producing animals than if ten cows were selected from a lower classification group.

TABLE 4

Chart showing average yield of 10 records selected at random from each classification group

Classification	Group 1 Aver. of 10 records	Group 2 Aver. of 10 records	Group 3 Aver. of 10 records	Group 4 Aver. of 10 records	Group 5 Aver. of 10 records	Group 6 Aver. of 10 records
Excellent	625	614	611	651	714	660
Very Good	628	621	620	564	574	576
Good Plus	572	627	569	665	563	588
Good	504	573	533	570	534	528
Fair	503	524	558	516	533	463

It has been suggested that the range in production within each classification group exceeds the differences between the various groups and that therefore the relationship between classification ratings and production is not significant. To determine the range in production of tested animals in each classification group, the following frequency table 5, was prepared.

TABLE 5

Frequency table showing distribution of records made by cows classified in various classification groups

Production divisions	Excellent	Very Good	Good Plus	Good	Fair
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
800 lbs. and over	8.87	6.29	7.02	3.03	2.63
700 to 799 lbs.	25.00	16.40	11.91	11.52	10.53
600 to 699 lbs.	27.42	23.37	20.83	20.20	13.16
500 to 599 lbs.	22.58	26.29	27.14	25.66	30.26
400 to 499 lbs.	12.10	22.25	23.81	25.25	27.63
Under 400 lbs.	4.03	5.39	9.29	14.34	15.79

Examination of this table does illustrate that there is a wide range in the producing ability of cows in each classification group and from the data in this table and the data in table 6, it is apparent that there is just as much variation in the production of cows classified "Excellent" as there is with cows in the other classification groups. The variation in production within each classification group is so marked, that a classification rating alone is of little value in estimating the producing ability of an individual cow.

TABLE 6

Classification groups	No. of cows classified with records	Production mean	Mean deviation of records	Standard deviation
Excellent	124	637.27	108.58	137.49
Very Good	445	590.11	100.45	139.68
Good Plus	840	574.37	118.15	150.27
Good	495	546.19	117.86	145.43
Fair	76	528.09	108.80	135.77

In comparing the "Excellent" animals with the cows classified as "Very Good" and doing this at random for the entire groups, it was found that in sixty-two per cent of the times, the "Excellent" cows had higher records than the "Very Good" cows. On the same basis, the chances are 53 out of 100 that a "Very Good" cow will have a higher record than a "Good Plus" cow. The chances are 56 out of 100 that a "Good Plus" cow will excel a "Good" cow and the possibility of a "Good" cow exceeding a "Fair" cow in production is 57 times out of 100 chances. The odds, on an "Excellent" cow having a higher record than a cow classified as "Fair" was found to be 73 to 27, or 73 times out of 100, the "Excellent" cow will exceed the "Fair" cow in production. It is apparent that a single animal cannot be selected from any one classification group with reasonable assurance that the animal selected from this group will be better or worse in production than an individual animal selected from any other classification group.

There are also a number of classified bulls with tested and classified daughters. The number is not large but the data are included since the inheritance of type and production is of such vital importance. There are 124 classified bulls, each having ten or more officially classified daughters. Twenty-one of these bulls have been rated "Excellent." The average classification score of their daughters is 84.51 per cent. There are 56 of this group of bulls rated "Very Good" and the average classification score of their daughters is 83.46 per cent. Forty-one of this group of bulls have been rated "Good Plus" and the average classification score of their daughters is 82.20 per cent. The remaining six bulls all classified "Good" have an average score on their daughters of 80.12 per cent. The correlation between the ratings of the 124 bulls and the average score of their daughters

was found to be $+.39 \pm .05$. This correlataion is significant in indicating some relationship between the conformation of a bull and the conformation ratings of his progeny. However, in this connection, it must be recalled that in most instances the good type bulls were bred to good type cows, whereas the poorer type bulls were probably used on cows of poorer conformation.

Next, 504 classified cows having classified dams were selected at random. The conformation ratings on the dams were compared with the conformation ratings on the daughters. The results are given in table 7.

TABLE 7

Frequency table—showing ratings on classified dams and classified daughters

Conformation ratings on dams	Percentage of daughters in each classification group					Average score of classified daughters
	Excel-lent	Very Good	Good Plus	Good	Fair	
68 Excellent dams	26.5	38.2	27.9	5.9	1.5	86.35
185 Very Good dams	7.6	37.3	38.4	15.1	1.6	84.13
162 Good Plus dams	3.7	21.0	46.9	25.9	2.5	82.34
81 Good dams	3.7	16.1	35.8	38.3	6.2	81.10
8 Fair dams	0	0	37.5	62.5	0	79.38

The average score of the 504 classified dams was 84.59, whereas the average score on the classified daughters was 83.29. The actual correlation coefficient between the ratings on the dams and the ratings on the daughters was calculated and was found to be $+.360 \pm .026$. This correlation coefficient is just slightly lower than the correlation between the conformation of the bulls and the bulls' daughters. It is significant in indicating a fair relationship between the conformation of a cow and the conformation of her daughters.

There are 128 classified bulls that have qualified as "A.J.C.C. Tested Sires," each having ten or more officially tested daughters. Twenty-one of these have been rated "Excellent" and their daughters have an average production of 620 pounds of fat. Fifty-five of this group of bulls have been classified "Very Good" and their daughters average 604 pounds of fat. There are forty-one of the "Tested Sires" that have been classified "Good Plus" and the records of their daughters average 611 pounds of butterfat. Then, there are ten "Tested Sires" classified "Good" whose daughters average 629 pounds of fat. The entire group of 128 classified "Tested Sires" have tested daughters averaging 610.78 pounds of fat. The correlation between the classification ratings on this group of 128 bulls and the records of their daughters was found to be $-.18 \pm .06$. There appears to be no relationship at all between the classification rating of a bull and the production of his progeny.

SUMMARY

From a practical breeding standpoint several conclusions may be drawn from the foregoing data, even though the correlations reported are not pronounced. It can be stated that there is some relationship between the conformation of a cow and her producing ability. It is equally obvious, however, that a high conformation score is not a reliable guarantee of superior producing capacity.

In breeding for conformation, there appears to be about the same correlation between the conformation of a cow and the conformation of her daughters as there has previously been found to exist between the producing ability of a cow and the producing ability of her daughters. In both instances, the correlations are significant but are hardly high enough to definitely insure the type of progeny which may be desired from dams of a certain conformation. It does appear that there is no relationship between the conformation of a bull and the producing ability of his daughters.

It can be concluded that type as determined by the score card and producing ability are not incompatible and that they can readily be combined in the same animal, yet to secure our ideals of type and production, breeders must continue to pay close attention to both of these essentials in their breeding programs.

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THERMODURIC BACTERIA IN PASTEURIZED MILK. II. STUDIES ON THE BACTERIA SURVIVING PASTEURIZATION, WITH SPECIAL REFERENCE TO HIGH-TEMPERATURE, SHORT-TIME PASTEURIZATION

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The recent interest in thermoduric bacteria in milk has been stimulated especially by the development of modern methods for high-temperature short-time pasteurization; the adoption of tryptone glucose extract milk agar as the standard medium for plate counts; agitation for the substitution of 32° C. (89.6° F.) instead of 37° C. (98.6° F.) for incubation of plate counts; and more stringent bacterial standards by certain Departments of Health. The first three tend to give higher counts with many milk supplies, which makes it difficult for the industry to meet the desires of the Health Departments for lower counts. Breed (1) has pointed out the desirability of concentrating efforts "on the extension of pasteurization as a blanket means of protection against milk-borne diseases and upon the eradication of diseases that may be transmitted to human beings from our milk-producing herds" before spending "an undue amount of energy and valuable time in getting some harmless types of bacteria out of our milk supplies. These bacteria are usually detected only because of the fact that they are difficult to kill during the pasteurization process." However, so long as pressure for very low bacteria counts is put on the industry by Health Departments, thermoduric bacteria present a serious problem, and their elimination from the milk supply must be undertaken.

A review of the literature on thermoduric bacteria in milk, which up to the present time has referred chiefly to those organisms surviving pasteurization by the low-temperature long-hold method, has recently appeared (2), and no attempt will be made to review it fully here.

A number of papers has been published (2, 3) showing that milk pasteurized by the high-temperature short-hold method usually has a higher bacteria count than does milk pasteurized by the low-temperature long-hold method. The question naturally arises as to what organisms are responsible for the difference in count. Eglinton and Yale (4) reported that yellow micrococci, found to originate in dirty farm utensils, were especially common on agar plates made from milk pasteurized by the high-temperature method, but there seem to have been no detailed quantitative studies of the

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differences in the flora of milk pasteurized by the two methods. Such a study was therefore undertaken.

EXPERIMENTAL

Fifteen lots of mixed milk from a large plant, with about 400 producers, were pasteurized by three methods, as follows:

1. In the laboratory in glass test-tubes at 143–144° F. (61.6°–62.2° C.) for 35 minutes, with about 5 minutes to heat and 5 minutes to cool.

2. In the laboratory in glass vials at 161° F. (71.5° C.) for 16 seconds. Five cubic centimeters of milk were placed in a 4 dram homeopathic vial, with the aluminum cap screwed down tight. The entire vial was completely immersed in a water bath maintained at 165° F. (75.9° C.) A thermometer dipped into the same amount of milk in a test-tube of the same diameter and same wall thickness as the vials. The milk reached 161° F. in about 2 minutes, when the rack holding the vials was removed from the water and held in the air for 16 seconds. The temperature rose to about 162° F. (72.2° C.) and then dropped to 160° F. (71.1° C.) during the 16 seconds, averaging very close to 161° F., after which the vials were dipped (but not immersed) into cold water. Expanding air in the vials prevented water from entering during the heating.

3. In a commercial plant, with a capacity of 22,000 pounds of milk per hour, at 161° F. for 16 seconds. In this apparatus, the temperature of the milk rose to 158° F. (70° C.) in 44 seconds, dropped to 143° F. (61.6° C.) in 4 seconds, finally reached 161° F. (71.5° C.) in 11 seconds, was maintained at 161° F. for 16 seconds, and was reduced to 40° F. (4.4° C.) in 35 seconds.

After cooling, plates were made from each of the three samples with tryptone glucose extract milk agar, and incubated 48 hours at 35°–37° C. Every colony from all three plates was inoculated into a tube of sterile litmus milk, incubated 48 or 72 hours at 35–37° C., and the reaction noted. Morphology of the cultures was determined microscopically. In all, 911 cultures from

TABLE 1

Litmus milk reactions of the bacterial flora of mixed plant milk pasteurized by three methods

Litmus milk reaction	Laboratory pasteurization at 143° F. for 35 minutes		Laboratory pasteurization at 161° F. for 16 seconds		Plant pasteurization at 161° F. for 16 seconds	
	Number	Per cent	Number	Per cent	Number	Per cent
Produce acid	182	93.3	173	65.8	205	45.3
Produce alkali	8	4.1	81	30.8	231	51.0
No change	4	2.1	6	2.3	11	2.4
Peptonize	1	0.5	3	1.1	6	1.3
Total	195	100.0	263	100.0	453	100.0

the 15 lots of milk (45 plates) were examined. Tables 1 and 2 show summaries of the results.

The significant features of table 1 are the decrease in the percentage of acid producers and the increase in the percentage of alkali producers in going from low-temperature laboratory pasteurization to high-temperature laboratory pasteurization to high-temperature commercial pasteurization. Table 2 shows an even sharper decrease in percentage of streptococci and increase in percentage of micrococci in going through the three methods of pasteurization.

TABLE 2

Morphological form of the bacterial flora of mixed plant milk pasteurized by three methods

Morphological form	Laboratory pasteurization at 143° F. for 35 minutes		Laboratory pasteurization at 161° F. for 16 seconds		Plant pasteurization at 161° F. for 16 seconds	
	Number	Per cent	Number	Per cent	Number	Per cent
Streptococci	134	68.7	78	29.7	80	17.7
Micrococci	46	23.6	156	59.3	346	76.4
Sarcinae	8	4.1	4	1.5	5	1.1
Rods	7	3.6	25	9.5	22	4.8
Total	195	100.0	263	100.0	453	100.0

In connection with table 1 it should be emphasized that the decrease in the *percentage* of acid producers in going from low-temperature laboratory to high-temperature laboratory to high-temperature plant pasteurization does not mean a decrease in *numbers* of these organisms surviving. The numbers of cultures of acid producers isolated from the milk pasteurized by the three methods were 182, 173 and 205, the differences being within what may be considered to be experimental error. However, the numbers of cultures of alkali producers isolated from the same samples were, respectively, 8, 81 and 231, a very significant increase.

The great increase in the numbers of alkali producers surviving high-temperature pasteurization, as compared with low-temperature pasteurization, might be considered by some as an undesirable feature of milk pasteurized by the high-temperature method. Milk containing these alkali producers might be expected not to sour normally, but might be more apt to develop undesirable flavors and odors as it gets old. The species of bacteria found are all undoubtedly entirely harmless except for this possibility of development of off-flavors if the milk were not properly cared for after delivery to the consumer. No evidence is at hand to indicate that such undesirable forms of spoilage actually do occur, and the distribution, over a period of 18 months, of large amounts of milk pasteurized in the commercial high-temperature short-time plant has not led to any particular complaint along this line.

From table 2 it appears that somewhat more streptococci occur in milk pasteurized at the low temperature than in that pasteurized at the high temperature. Examination of table 4, where numbers per cubic centimeter of the various types of organisms are given for each sample separately, reveals that samples 12 and 13 account for this. Data are not available to show why these two samples contained so many more than the average number of streptococci capable of surviving low-temperature pasteurization. It might be that these two samples contained streptococci able to survive low-temperature pasteurization better than high-temperature, or that growth of *Streptococcus thermophilus* occurred during the 35-minute holding, but neither explanation seems very probable.

Since the alkali-producing bacteria make up such a large percentage of the flora of the milk pasteurized in the commercial apparatus at 161° F. (71.5° C.) for 16 seconds in this particular plant, it is of interest to analyze further the data on the commercially-pasteurized milk. Table 3 shows monthly averages over a five-month period covering the counts of all organisms, of alkali-producing organisms, and of non-alkali-producing organisms. The total count increased gradually from April through August and it is obvious that this increase can be attributed quite largely to the alkali-producing organisms, except for the month of August.

TABLE 3

Numbers per cubic centimeter of various types of organisms surviving commercial high-temperature pasteurization

Month	Number of differential counts made	Monthly average of counts		
		Total organisms	Alkali producers	Non-alkali producers
April	1	14,000	None	14,000
May	2	20,000	5,000	15,000
June	7	31,000	16,000	15,000
July	4	34,000	24,000	10,000
August	1	45,000	14,000	31,000

Table 4 shows, for each of the 15 lots of milk, the numbers per cubic centimeter of various types of organisms surviving when the milk was pasteurized by the three methods. The increase in the number of alkali producers in going from laboratory low-temperature to laboratory high-temperature to plant high-temperature pasteurization accounts for most of the increase in total count, leaving the number of non-alkali-producing organisms nearly constant in the milk pasteurized by all three methods. However, examination of the two right-hand columns shows that changes in the flora other than an increase in the number of alkali-producing organisms occur when changes in the method of pasteurization are made. The number of streptococci (all of which were acid-producers) decreases, and the number of non-alkali-producers other than streptococci increases, when a change from low-

TABLE 4

Number per cubic centimeter of various types of organisms surviving in milk pasteurized by three methods

Pasteurization method	Sample number	Numbers of organisms				
		Total organisms of all kinds	Organisms producing alkali	Organisms not producing alkali		
				Total	Strepto-cocci	Organisms other than strepto-cocci
143° F. for 35 minutes in the laboratory	1	10,000	3,000	7,000	4,000	3,000
	2	10,000	0	10,000	2,000	8,000
	3	6,000	0	6,000	1,000	5,000
	4	11,000	1,000	10,000	7,000	3,000
	5	15,000	0	15,000	12,000	3,000
	6	8,000	0	8,000	7,000	1,000
	7	3,000	0	3,000	3,000	0
	8	6,000	1,000	5,000	3,000	2,000
	9	5,000	0	5,000	3,000	2,000
	10	1,000	0	1,000	1,000	0
	11	5,000	0	5,000	1,000	4,000
	12	40,000	0	40,000	39,000	1,000
	13	32,000	3,000	29,000	29,000	0
	14	21,000	0	21,000	13,000	8,000
	15	22,000	0	22,000	3,000	19,000
	Average	13,000	533	12,466	8,500	3,933
161° F. for 16 seconds in the laboratory	1	9,000	2,000	7,000	2,000	5,000
	2	9,000	0	9,000	2,000	7,000
	3	24,000	7,000	17,000	9,000	8,000
	4	13,000	2,000	11,000	5,000	6,000
	5	25,000	3,000	22,000	15,000	7,000
	6	20,000	7,000	13,000	5,000	8,000
	7	16,000	9,000	7,000	2,000	5,000
	8	37,000	22,000	15,000	12,000	3,000
	9	19,000	7,000	12,000	5,000	7,000
	10	8,000	0	8,000	6,000	2,000
	11	9,000	3,000	6,000	2,000	4,000
	12	12,000	8,000	4,000	1,000	3,000
	13	17,000	8,000	9,000	5,000	4,000
	14	10,000	2,000	8,000	2,000	6,000
	15	35,000	1,000	34,000	5,000	29,000
	Average	17,533	5,400	12,133	5,200	6,933
161° F. for 16 seconds in the plant	1	14,000	0	14,000	4,000	10,000
	2	20,000	4,000	16,000	1,000	15,000
	3	20,000	6,000	14,000	7,000	7,000
	4	23,000	6,000	17,000	7,000	10,000
	5	43,000	13,000	30,000	24,000	6,000
	6	26,000	12,000	14,000	7,000	7,000
	7	34,000	18,000	16,000	4,000	12,000
	8	45,000	33,000	12,000	4,000	8,000
	9	29,000	16,000	13,000	2,000	11,000
	10	16,000	13,000	3,000	2,000	1,000
	11	28,000	12,000	16,000	7,000	9,000
	12	27,000	23,000	4,000	0	4,000
	13	56,000	41,000	15,000	4,000	11,000
	14	27,000	20,000	7,000	0	7,000
	15	45,000	14,000	31,000	7,000	24,000
	Average	30,200	15,400	14,800	5,333	9,467

temperature to high-temperature pasteurization is made. The increase in the last-mentioned group consists largely of acid-producing micrococci. The unusually large numbers of streptococci surviving low-temperature pasteurization in samples 12 and 13 have already been discussed.

All of the cultures obtained from three of the lots of milk pasteurized in three ways (or from nine plates in all) were identified, using the classification of Sherman (5) for the streptococci and of Hucker (6) for the micrococci. Table 5 shows the results. Here again, we see the decrease in percentage of streptococci and the increase in percentage of micrococci observed in table 2 in going from low-temperature laboratory pasteurization to high-temperature laboratory pasteurization to high-temperature commercial pasteurization. The two species of streptococci (5) and six of the nine species of micrococci (7, 8, 9) encountered have previously been shown by other workers to be capable of surviving low-temperature long-time pasteurization. The three species of micrococci not previously known to be thermoduric (*M. flavus, M. freudenreichii* and *M. caseolyticus*) were found only in the milk pasteurized in the laboratory at 161° F. (71.5° C.) for 16 seconds, and the three combined made up only 9.6 per cent of the cultures identified from such milk.

TABLE 5

Species of bacteria surviving in three different lots of mixed plant milk pasteurized by three methods

Specific name	Laboratory pasteurization at 143° F. for 35 minutes		Laboratory pasteurization at 161° F. for 16 seconds		Plant pasteurization at 161° F. for 16 seconds	
	Number of cultures	Per cent of cultures	Number of cultures	Per cent of cultures	Number of cultures	Per cent of cultures
M. candidus	2	8.7	11	26.2	31	35.2
S. bovis	9	39.2	12	28.6	15	17.1
S. thermophilus	6	26.1	4	9.5	14	15.9
M. epidermidis	1	4.3	4	9.5	13	14.8
M. luteus	1	4.3	3	7.1	9	10.2
M. varians	3	13.1	3	7.1	3	3.4
M. conglomeratus	1	4.3	1	2.4	2	2.3
M. flavus	......	....	2	4.8	......	....
M. albus	......	....	......	....	1	1.1
M. freudenreichii	......	....	1	2.4	......	....
M. caseolyticus	......	....	1	2.4	......	....
Total	23	100.0	42	100.0	88	100.0
Micrococci	8	34.7	26	61.9	59	67.0
Streptococci	15	65.3	16	38.1	29	33.0

It seemed desirable to determine if the milk from individual farms would give a flora similar to that obtained from the mixed plant milk. Therefore, samples of milk from 49 farms which had at some previous time shown high

thermoduric counts were pasteurized in the laboratory at 161° F. (71.5° C.) for 16 seconds, plate counts were made and all of the 484 colonies from all of the plates were inoculated into sterile litmus milk and examined as in the case of the mixed plant milk. The data are summarized in tables 6 and 7. Results are very similar to those obtained in the examination of the mixed plant milk (tables 1 and 2), except that the percentages of alkali producers and of streptococci were somewhat lower. These particular samples were studied in August. Examination of the bottom line of table 3 will show that the single sample of commercially-pasteurized milk examined in August also contained a smaller percentage of alkali producers than usual, only 31.1 per cent as compared with an average of 53.2 per cent in the other 14 lots of commercially-pasteurized milk examined. This single lot of mixed plant milk examined in August contained only 1 alkali producer out of a total of 35 cultures examined (or 2.9 per cent) when pasteurized in the laboratory at 161° F. for 16 seconds. The causes of these fluctuations have not been determined.

TABLE 6

Litmus milk reaction of bacteria surviving in the milk of 49 producers pasteurized in the laboratory at 161° F. for 16 seconds

Litmus milk reaction	Number of cultures	Per cent of cultures
Produce acid	340	70.3
Produce alkali	85	17.6
Produce no change	38	7.8
Peptonize	21	4.3
Total	484	100.0

TABLE 7

Morphology of bacteria surviving in the milk of 49 producers pasteurized in the laboratory at 161° F. for 16 seconds

Morphological form	Number of cultures	Per cent of cultures
Micrococci	384	79.3
Streptococci	36	7.4
Sarcinae	39	8.1
Rods	25	5.2
Total	484	100.0

Thirty-eight of the 484 cultures were identified. The cultures chosen for identification were from the predominant group, based on litmus milk reaction and morphology, making up the bulk of the flora in the pasteurized milk from each farm where the thermoduric count was high. Names of these organisms are given in table 8. The species are the same as those found in the mixed plant milk pasteurized in the same way, except that *S. bovis* was not encountered in the farm samples.

The next step in tracing the source of the thermoduric bacteria in milk pasteurized at 161° F. for 16 seconds consisted of checking equipment on

TABLE 8

Names and approximate proportions of the typical organisms found in the milk of individual producers giving high counts after pasteurization in the laboratory at 161° F. for 16 seconds

Specific name	Number of cultures	Per cent of cultures
M. candidus	9	23.7
S. thermophilus	6	15.8
M. epidermidis	1	2.6
M. luteus	7	18.4
M. varians	2	5.3
M. flavus	1	2.6
M. conglomeratus	7	18.4
M. freudenreichii	3	7.9
M. caseolyticus	2	5.3
Total cultures	38	100.0
Micrococci	32	84.2
Streptococci	6	15.8

19 of the 49 farms referred to above. The worst milk cans that were found on the farms, from the standpoint of rust and cracked seams, were rinsed with two quarts of sterile milk. Two quarts of sterile milk were also used to rinse strainers and pails (combined) on 17 farms. In the case of the 11 milking machines examined, four quarts of sterile milk were drawn through the teat cups and tubes into the milking machine pails. Samples of the rinsings were pasteurized in the laboratory at 161° F. for 16 seconds, and plated at three dilutions, so as to get plates with few enough colonies for easy picking into litmus milk. The results of the examination of 520 cultures so isolated are shown in tables 9 and 10. These data differ somewhat from those on the mixed plant milk pasteurized at the same temperature in that there are fewer alkali producers and fewer streptococci. In this respect the cultures obtained from the milk of individual producers resembled those obtained from farm equipment more closely than either of these two groups of cultures resembled those obtained from mixed plant milk. In all three groups of cultures, micrococci predominated. The utensils also were examined in August.

TABLE 9

Litmus milk reaction of the bacteria isolated from utensils on 19 farms and capable of surviving pasteurization in the laboratory in milk at 161° F. for 16 seconds

Litmus milk reaction	Milk cans on 7 farms		Milking machines on 11 farms		Strainers and pails on 17 farms		Total for all utensils	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Produce acid	40	58.8	232	95.10	178	85.6	450	86.5
Produce alkali	10	14.7	2	0.80	7	3.4	19	3.7
Produce no change	16	23.5	5	2.05	17	8.2	38	7.[illegible]
Peptonize	2	3.0	5	2.05	6	2.8	13	2.[illegible]
Total	68	100.0	244	100.0	208	100.0	520	10[illegible]

TABLE 10

Morphology of the bacteria isolated from utensils on 19 farms and capable of surviving pasteurization in the laboratory in milk at 161° F. for 16 seconds

Morphology	Milk cans on 7 farms		Milking machines on 11 farms		Strainers and pails on 17 farms		Total for all utensils	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Streptococci	5	7.4	8	3.2	13	6.3	26	5.0
Micrococci	37	54.4	171	70.1	156*	75.0	364	70.0
Sarcinae	0	0.0	58	23.8	23	11.0	81	15.6
Rods	26	38.2	7	2.9	16	7.7	49	9.4
Total	68	100.0	244	100.0	208	100.0	520	100.0

* Of the 156 micrococci isolated from strainers and pails, 60, or 28.8 per cent of the 208 cultures, grew in definite tetrads (not cubes). This was the largest group of these organisms found.

Seventeen of what appeared to be the most common cultures obtained from strainers and pails and from milking machines were identified, as shown in table 11. All of the 6 species found among these 17 cultures had previously been found in the mixed plant milk and in the milk from individual farms, pasteurized in the laboratory at 161° F. for 16 seconds. All but *M. freudenreichii* (of which 1 culture only was encountered) had been previously reported by other workers as being capable of surviving pasteurization by the low-temperature long-time method (7, 8, 9).

TABLE 11

Names and approximate proportions of the bacteria found in equipment on dairy farms and capable of surviving laboratory pasteurization at 161° F. for 16 seconds

Specific name	Strainers and pails		Milking machines		Total	
	Number of cultures	Per cent of cultures	Number of cultures	Per cent of cultures	Number of cultures	Per cent of cultures
M. varians	2	25.0	3	33.3	5	29.4
M. luteus	2	25.0	3	33.3	5	29.4
M. candidus	1	12.5	2	22.2	3	17.6
S. thermophilus	1	12.5	1	11.1	2	11.8
M. conglomeratus	1	12.5	...	....	1	5.9
M. freudenreichii	1	12.5	...	....	1	5.9
Total	8	100.0	9	99.9	17	100.0
Micrococci	7	87.5	8	88.8	15	88.2
Streptococci	1	12.5	1	11.1	2	11.8

SUMMARY AND CONCLUSIONS

The higher bacteria counts occurring in milk pasteurized by the high-temperature short-hold method as compared with the low-temperature long-hold method are largely due to the ability of certain species of micrococci to

survive the former method of pasteurization in greater numbers. The most common species of micrococci among those found in the milk pasteurized at high temperature are *M. candidus*, *M. epidermidis*, *M. luteus* and *M. varians*, although five other species were encountered less frequently. These micrococci make up the predominant thermoduric flora of dirty milking machines, strainers and pails on farms, and about half of the thermoduric organisms isolated from milk cans. However, milk cans at the plant where this work was done were not an important source of thermoduric bacteria.

The work of Harding and Wilson (10) and of Alice Breed (11) indicates that micrococci make up about 75 per cent of the flora of the normal cow's udder. They studied in all 226 cultures and found that six of the seven species encountered in commercially pasteurized milk in the work reported here made up about 60 per cent of the micrococci of the udder, or over 45 per cent of the total flora of normal udders. The only species encountered in this work not found in the udder by these previous workers was *M. caseolyticus*, and only 3 cultures of this organism were encountered among a total of 153 cultures of micrococci identified. The principal source of bacterial contamination of the rubber tubes of a milking machine is probably the milk itself. Moreover, many of these species of micrococci can survive inefficient hot-water sterilization just as they can survive pasteurization. Robertson (12) reports that many of them apparently also can survive sterilization by chlorine sterilizers and by salt brine. All this explains very well the source of the thermoduric micrococci in the milk. They originate in the udder and grow in improperly cleaned dairy farm utensils.

Workman has reported (13) that micrococci occur in the soil, in the stable air, and on the skin of cows. All of these sources doubtless contribute somewhat to the original contamination of dairy farm utensils and of the milk itself. However, it must be emphasized that dust, dirt from the skin of the cow, and the udder itself contribute relatively very small numbers of organisms to the milk (14, 15). Only when dairy farm utensils are improperly cleaned so as to offer a place where the micrococci can multiply does the problem of thermoduric bacteria become a serious one.

The data presented here were obtained at a single large milk plant, with about 400 producers. Whether similar results would be obtained with other milk supplies can only be determined by further investigation, now under way.

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THE USE OF NEMBUTAL ANESTHESIA IN MILK SECRETION STUDIES*

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Since the development of reliable methods for obtaining arterial and mammary venous blood samples for the study of milk secretion in normal intact animals, the avoidance of excitement during sampling has been shown to be one of the prime requisites for securing results of physiological value. Graham, Kay, and McIntosh (1) showed that valid results could be obtained with cows if the venous sampling was completed within two minutes and the arterial sampling within four minutes or less after first touching the animal. They reported that there was no detectable change in arterial blood if the above time limits were not exceeded. However, they stated that even if these precautions are observed, serious disturbance of the animal will decrease the value of the results. Blackwood and Stirling (2) detected no significant changes in the iron content of the blood in traversing the mammary gland. Graham *et al.* (3) reported changes in hemoglobin concentration averaging less than one per cent between arterial and venous samples of goat blood. On the other hand, Petersen and Boyd (4) and Shaw and Petersen (5) reported that large changes in blood volume, as indicated by differences in the hemoglobin level, occur frequently in arterio-venous samplings from cows, and they correlated these changes with the degree of excitation of the animal. Attempts to correct the analytical values for blood volume changes led to untenable results, and they recommended that only those samples showing little or no change in blood volume be used in studying milk secretion. Further difficulties in the study of certain phases of milk secretion in the normal intact goat are shown by the report (6) that the total nitrogen of both arterial and venous blood and plasma fluctuates sufficiently from moment to moment to mask, in some cases, the actual uptake of nitrogen by the mammary gland that would be involved in milk secretion.

In view of the variation in results encountered in sampling normal animals it was decided to test the feasibility of securing arterio-venous differences with the animal under the influence of a general anesthetic. Nembutal was chosen for this purpose because it had been shown by Hrubetz and Blackberg (7) to have no effect on the level of blood sugar during the period of anesthesia. Hafkesbring *et al.* (8) reported that dogs anesthetized

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with nembutal once a week for 6 to 8 weeks show no significant change in blood or urine composition.

In order to determine the effect of the anesthetic on the rate of milk secretion, two goats were nembutalized, following which they were milked out completely with the aid of one-half ml. of pituitrin given intravenously to eject the last of the milk. The anesthesia was maintained for 3½ and 4 hours, respectively, by periodic injections of small amounts of nembutal. The goats were then milked exactly as at the start of the period. The normal hourly milk production previous to the experiment was computed from the average daily yield of the 4 previous days. It was found (table 1) that the anesthesia had no effect on the rate of milk secretion, since the hourly milk yield in both cases was within one-half ml. of the normal. Suitable control values for the milk analysis were not obtained, but the constituents of the experimental milk samples were well within the range for normal goat milk.

TABLE 1

The effect of nembutal anesthesia on the yield and composition of milk

	Milk production	
	Goat No. 836	Goat No. 438
	ml.	*ml.*
Normal		
Milk per hour	27.45	39.0
Experimental		
Total yield	108.0	135.0
Milk per hour	27.0	38.5
Hours on experiment	4.0	3.5

Composition of milk produced under anesthesia

	Fat	Lactose	Total nitrogen	Calcium
	per cent	*gms. per 100 ml.*	*mgm. per cent*	*mgm. per cent*
Goat No. 836	6.9	4.73	523	151.5
Goat No. 438	4.4	5.12	452	148.8

In preparation for drawing blood samples the goats were given an intraperitoneal injection of 25 to 30 grains of nembutal for a mature animal weighing 70 to 90 pounds. After allowing 15 to 30 minutes for the anesthetic to take effect the samples were drawn by the method of Graham, Gomez and Turner (9) into flasks coated with equal amounts of sodium fluoride and potassium oxalate. Care was taken to assure free and unimpeded respiration of the animal during the entire procedure since any interference with respiration was found to have a disturbing effect on the oxygen and carbon dioxide levels of the blood. In order to prevent excess saliva from collecting in the respiratory passages and hampering respiration the animal was placed with its head at a lower level than the body, and its tongue drawn out of the side of its mouth to provide drainage.

TABLE 2

Arteriovenous differences of blood sugar of lactating goats under nembutal anesthesia

Exp. No.	Goat No.	Blood sugar			
		Arterial	Venous	Difference	Hemoglobin difference
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*per cent*
41	231	41.56	27.68	14.88	+2.00
46	231	39.44	27.93	11.52	0
47	231	41.36	25.52	15.84	−0.65
57	231	36.25	24.56	11.75	+0.98
59	231	43.75	32.75	11.00	−1.79
60	231	44.75	27.75	17.00	−0.49
71	231	39.75	27.00	12.75	−0.60
74	231	49.60	41.50	8.10	+0.96
62	842	40.25	32.00	8.25	+0.17
63	842	49.00	38.00	10.60	+1.80
65	842	42.00	33.25	8.75	+0.68
66	842	44.50	32.75	11.75	−1.25
69	842	53.50	40.00	13.50	+0.86
70	842	50.00	39.50	10.50	+1,44
72	842	53.25	41.50	11.75	−0.15
76	842	54.00	42.75	11.25	+0.40
77	842	58.00	47.75	10.25	+1.60
78	842	56.80	46.00	10.80	0
79	842	58.75	49.80	9.95	+1.11
80	842	56.80	48.00	8.80	+1.52
81	842	51.00	43.50	7.50	+2.57
82	842	51.75	43.50	8.25	−1.50
42	836	50.93	37.52	13.41	+1.25
84	836	49.20	33.75	15.45	−1.13

Blood sugar was determined by the Shaffer-Somogyi method (10) and total hemoglobin by the photoelectric method of Evelyn and Malloy (11).

The data show that there is a consistent uptake of blood glucose by the lactating mammary gland under the given conditions (table 2). The differences in hemoglobin concentration average only one per cent, and, in the majority of cases, are within the experimental error of the analytical method used. A less extended series of analyses on blood lactic acid as well as on the neutral fat plus cholesterol fraction of the blood plasma indicate that these substances are also taken up by the lactating mammary gland in appreciable amounts under the experimental conditions described. Further data (unpublished) on the respiratory quotient of the mammary gland of the nembutalized goat show a constant figure, varying but little from the value of 1.09. It thus appears that the rather wide variations in the respiratory quotient of the intact mammary gland previously encountered by Graham *et al.* (12) and Shaw (13) may be eliminated to a large extent by nembutal anesthesia.

SUMMARY

It was shown that goats maintained under nembutal anesthesia continue to secrete milk of normal composition at the normal rate. Comparisons

between arterial and mammary venous blood samples drawn from goats anesthetized with nembutal showed that the uptake of milk precursors continues under these conditions. In view of these results it was pointed out that the adverse effects caused by excitement of the animal while drawing arterial and venous blood samples may be eliminated by the administration of nembutal.

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VARIATIONS IN BULL SEMEN AND THEIR RELATION TO FERTILITY*

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With the wide-spread use of artificial breeding in dairy cattle the characteristics of the semen of the dairy sire and their relation to fertility have become of great interest. The accurate evaluation of the fertility and longevity of a sample of semen at the time of collection would be a useful tool in artificial insemination circles. However, no single satisfactory method of making such evaluation has yet been presented. Various methods have been proposed for determining the fertility of the bull from routine examinations of his semen and the principles of such methods are generally applied in the evaluation of a single sample.

One of the earliest requirements of fertile semen was motility. Donham *et al.* (6) found that semen below normal in motility was less than half as effective in producing conception as was semen of normal, 90 per cent or more motility. Although motility of the spermatozoa was considered important by Williams, W. W. (15), Williams, W. L. (14), Lagerlöf (10), Sciuchetti (13) and others, they did not consider motility alone a reliable index of fertility. Davis (4) believed initial motility of the spermatozoa to be the best single evidence of viability. The duration of motility in stored semen was reported by William, W. L. (14) and Comstock (2) to be a good index of fertility. Comstock (2) and Comstock and Green (3) also reported that the rate of glycolysis of fresh ram semen was proportional to viability of semen and proposed evaluating the semen from its rate of glycolysis in conjunction with its density and percentage of abnormal spermatozoa. Walton and Edwards (18) reported that the initial respiration rate of bull semen was proportional to the breeding efficiency of the bulls.

Examinations of the stained spermatozoa have been reported by many investigators to be helpful in determining fertility. There is considerable disagreement upon the number of abnormal spermatozoa compatible with good fertility, however. Williams and Savage (16, 17) found no highly efficient breeding bulls with over 17 per cent abnormal spermatozoa. Lagerlöf (10) stated that bulls of good fertility produced not more than 18 per cent abnormal spermatozoa. Kuhne (9), Generales (7), and Sciuchetti (13) stated that fertile bulls produced not over 25 per cent abnormal spermatozoa. Some of this disagreement may be due to the differences in opinion as to what constitutes abnormal spermatozoa. This method, however, is subject

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to the same criticisms as motility determinations in that it is only a partially reliable index of fertility. Williams and Savage (16) found demonstrable morphological changes in the semen of only one-third of the poor breeding bulls they examined. Biometrical study of the spermatozoan-head length variation was shown to be indicative of the bull's fertility by Lagerlöf (10), Williams and Savage (16), and Savage, Williams and Fowler (11). The coefficient of variation of head lengths of spermatozoa from bulls of good fertility was not over 4.0.

Another property of the semen which may have some bearing on its fertility is pH. Schneerson (12) and Kuhne (9) reported that pH of the semen should be below 6.60; however, Davis and Williams (5) found that a large majority of the ejaculates from 11 fertile bulls was alkaline in pH.

The properties of semen produced by a bull are not constant. Hence the evaluation of his fertility does not insure the fertility of every sample of semen he produces. This variation in properties of semen has been reported by Lagerlöf (10), Donham and co-workers (6), Sciuchetti (13), Davis and Williams (5), Bartlett and Perry (1) and others. The variation in performance of different bulls has also been pointed out.

This study was made to determine the normal variation in certain properties of semen collected regularly from several bulls of varying degrees of fertility and to note the relation of fertility of the semen to its measurable characteristics.

MATERIALS AND METHODS

The ten bulls included in this study were all in regular service at the University of Missouri dairy herd. They ranged in age from one and one-half to nine years. The Jersey, Guernsey and Holstein-Friesian breeds were represented. Care and management of all bulls were practically the same.

The breeding records of each bull included all services, both natural and artificial, to cows with good breeding histories along with the number of conceptions secured. Pregnancies were determined by examination and by failure of the cows to come in heat after service. The period included in the tabulation of the breeding records was the year prior to April 1, 1940, or such portion of that time as the bull had been in service in the University of Missouri herd.

The semen was collected by use of the artificial vagina as described by Herman and Ragsdale (8). Prior to January, 1940, semen was collected intermittently, but thereafter collections were made regularly twice a week, with the exception of bulls 8, 52, 53, and 47 from which samples were obtained at varying intervals. The bulls were handled as nearly as possible in the same manner at each collection. It was tried to prevent their mounting until they were well stimulated as evidenced by drippings from the sheath. In some cases this was not possible. Often if a thin, watery sample

was secured, a second ejaculate collected immediately was of normal consistency. The semen was collected in a warm collecting tube and not cooled before the initial examinations were made. This varied from 10 to 30 minutes after collection. At that time the semen sample was divided and part of it was stored undiluted in a refrigerator at 40° Fahrenheit. The rest was kept for observation and experimentation.

Notes were made as to the appearance and consistency of the fresh semen, and the volume of each ejaculate was recorded. The concentration of spermatozoa was determined by use of a haemacytometer and pH was determined by means of a Beckman glass electrode pH meter. A smear was made of each sample of semen and stained five to ten minutes with three per cent aqueous Rose Bengal solution. The stained spermatozoa were then examined at 1075 × magnification for morphological abnormalities. The semen was rated for motility initially and at intervals during storage. All motility examinations were made at 100° Fahrenheit in a microscope stage incubator using a 250 × magnification. Motility was rated according to its vigor at 0 to 5. The very best motility was rated 5. No motility was 0. Semen in which the motion was largely weak and oscillatory and in which less than 40 to 50 per cent of the spermatozoa seemed to be in motion was rated 1. Ratings of 2, 3, and 4 represented progressive degrees of spermatozoan activity between the ratings of 1 and 5.

OBSERVATIONS

The results of examinations of 256 samples of semen collected from the 10 bulls were tabulated, and the average values for each characteristic of the semen of each bull were calculated. These averages are given in table 3 along with the bull's breeding ratios. Tables 1 and 2, showing records of semen examinations of two bulls, are presented to illustrate the wide variation in properties of the semen of the same bulls at different ejaculates. Similar records were prepared for all the bulls, but the data were too voluminous to be presented here in their entirety. The records of these bulls are representative of variations shown in records of the other bulls. The records show wide variability in nearly all characteristics of the semen of these two bulls, though semen of bull 49 was less variable than that of bull 50.

The initial motility rating showed the least variation of the characteristics of the semen examined. With the individual bulls it was either uniformly good or uniformly poor. Two bulls which consistently produced semen of poor initial motility were of poor fertility, but not all bulls which produced semen of good initial motility were of high fertility. For most bulls a very large percentage of the spermatozoa had the power of vigorous motility at ejaculation. The duration of vigorous motility under storage conditions was quite variable, however, as is shown in the column listing the maintenance of 2 motility. The hours which a motility rating of 2 or better

TABLE 1

Record of semen examinations of bull No. 50

Date of collection	Appearance of semen	Volume	Concentration	pH	Initial motility rating	Maintenance of 2 motility	Abnormal spermatozoa per 1000				
							Total	Tail-less	Coiled tails	Pyriform	Other abnormals
		cc.	*1000/cu.mm.*			*hours*					
1–31–40	Normal	5.5	1248	5.95	5	144	225	15	6	186	18
2– 5–40	Thin	3.0	544	6.25	4	120	357	18	246	87	6
2– 8–40	Thin	5.7	464	7.00	5	24	165	78	39	27	21
2–15–40	Normal	8.0	752	7.80	3	0	90	12	21	45	12
2–19–40	Normal	4.4	972	6.25	5	72	57	12	15	30	0
2–22–40	Normal	4.5	192	6.45	4	168	414	15	234	144	21
2–27–40	Normal	0.5	1280	6.65	3	......	246	21	135	81	9
2–29–40	Thin	3.2	680	6.70	5	6	117	18	33	63	3
2–29–40*	Normal	7.5	1040	6.40	5	48	126	12	36	75	3
3– 4–40	Thin	4.5	592	6.45	5	16	129	12	72	45	0
3– 5–40	Clear	2.5	56	6.55	2	0	135	21	24	66	24
3– 5–40*	Thin	5.0	600	6.55	5	144	246	21	105	114	6
3– 7–40	Normal	6.0	1040	6.40	5	120	213	3	33	168	9
3–11–40	Normal	4.0	896	6.40	4	24	426	63	87	276	0
3–14–40	Normal	4.0	724	6.55	4	72	249	24	69	147	9
3–14–40*	Normal	9.0	800	6.50	4	96	294	18	135	141	0
3–18–40	Normal	2.5	1096	6.20	5	144	234	30	72	129	3
3–21–40	Thin	4.0	672	6.55	5	24	228	15	93	111	9
3–25–40	Normal	2.6	1160	6.35	2	16	330	90	72	168	0
3–28–40	Normal	3.0	1160	6.20	5	24	183	24	36	123	0
3–28–40*	Normal	7.5	704	6.30	5	24	237	99	12	126	0
4– 1–40	Clear	3.5	160	6.50	3	0	141	90	15	30	6
4– 5–40	Thin	3.0	144	6.50	1	0	126	54	6	66	0
4– 5–40*	Thin	5.2	360	6.45	5	24	120	51	21	48	0
4– 9–40	Thin	3.0	576	......	5	20	231	15	51	132	33
4–13–40	Thin	3.0	304	6.60	4	24	273	48	18	207	0
4–13–40*	Normal	5.0	856	6.45	5	144	312	126	27	159	0
4–17–40	Normal	4.0	608	......	5	120	192	51	36	102	3
4–20–40	Normal	8.5	960	6.20	5	168	162	36	30	90	6
4–30–40	Normal	6.5	1104	6.35	5	168	201	27	12	162	0
Averages		4.62	724.8	6.48	4.27	67.4	215	37	60	111	7

* Second ejaculate.

TABLE 2

Record of semen examinations of bull No. 49

Date of collection	Appearance of semen	Volume	Concentration	pH	Initial motility rating	Maintenance of 2 motility	Abnormal spermatozoa per 1000				
							Total	Tail-less	Coiled tails	Pyriform	Other abnormals
		cc.	1000/cu.mm.			hours					
1–10–40	Normal	4.5	520	6.40	5	96	129	39	84	3	3
1–13–40	Thick	3.5	608	6.15	5	48	237	27	186	15	9
1–17–40	Normal	4.6	944	6.25	5	6	87	30	48	3	6
1–20–40	Thick	7.0	1744	6.60	3	6	48	9	33	3	3
1–24–40	Normal	3.7	992	6.70	3	240	372	6	366	0	0
1–27–40	Normal	3.5	336	6.40	5	72	96	42	36	6	12
1–31–40	Normal	11.0	728	6.45	2	96	546	54	489	0	3
2– 3–40	Thick	5.0	1624	5.95	5	144	129	33	93	3	0
2– 6–40	Normal	4.5	1272	6.10	5	72	84	39	39	3	3
2–10–40	Thick	3.5	880	6.20	5	120	72	39	24	3	6
2–13–40	Normal	3.1	816	6.40	5	120	114	45	66	0	3
2–17–40	Normal	1.7	1000	6.60	5	168	48	15	30	0	3
2–20–40	Normal	3.9	816	6.20	5	216	78	27	48	0	3
2–24–40	Normal	3.5	728	5.95	5	160	69	12	48	6	3
2–24–40*	Normal	4.0	816	6.05	5	144	84	21	57	6	0
2–27–40	Normal	3.3	1296	6.05	5	144	114	60	51	0	3
3– 2–40	Normal	2.8	800	6.25	5	144	126	18	108	0	0
3– 2–40	Normal	4.0	1280	6.20	5	168	30	18	6	3	3
3– 5–40	Normal	3.0	1344	6.25	5	48	51	9	39	3	0
3– 9–40	Thick	4.5	960	6.20	5	120	90	18	63	3	6
3–12–40	Normal	5.0	1140	6.10	5	144	117	33	78	0	6
3–19–40	Thick	6.0	1408	6.20	5	72	63	3	45	12	3
3–26–40	Normal	4.0	1640	6.25	5	72	144	0	144	0	0
3–30–40	Normal	5.0	880	6.10	5	72	87	36	45	0	6
4– 2–40	Normal	3.7	616	6.40	5	72	60	18	42	0	0
4– 6–40	Normal	6.0	2032	6.05	5	168	36	3	15	6	12
4– 9–40	Normal	6.5	1392	6.25	5	288	81	6	69	6	0
4–13–40	Normal	4.0	1064	6.30	5	96	60	27	24	9	0
Averages		4.46	1059.86	6.25	4.75	118.4	116	25	85	3	4

* Second ejaculate.

was maintained were determined from the last time at which a motility rating of 2 was observed. Thus if the motility rating at the second examination were 1, the time motility of 2 was observed would be only the initial examination or 0 hours. Although the duration of 2 motility seemed to be quite variable, apparently irrespective of other properties of the semen, such as pH, percentage of abnormal spermatozoa, and concentration, it was roughly correlated with the initial motility of the semen of any one bull. This relationship is shown quite well in the semen of bull 50. Though not all the samples which had an initial motility rating of 5 survived for a long period, none of those which rated motility of 3 or less initially had a motility rating above 1 at 24 hours. This did not hold true for all samples from the bulls (as notice two samples from bull 49 rating motilities of 2 and 3 which kept 2 motility for 96 and 240 hours, respectively) but in a large number of cases the semen of any one bull which was markedly below his normal in initial motility was also below his normal in survival with good motility.

Wide variations were observed in the percentage of abnormal spermatozoa in semen. Except for the first few collections from bull 49, the morphological variations in his semen were relatively slight. On the other hand, the semen of bull 50 varied greatly in percentage of abnormal spermatozoa, as is shown in table 1. For most of the bulls the variations in abnormal spermatozoa were largely due to a relatively large increase of the predominating type of abnormality rather than the same increase of all types. Thus most of the variations for bull 49 were due to an increase in coiled tails, but for bull 50 the increases were mainly pyriform heads.

The pH of semen samples from the same bull was subject to considerable variation. The variations in pH of the semen of bull 49 were not as great as those in semen of bull 50. The bulls whose semen varied most in pH (such as bull 50) also produced semen which was quite variable in consistency and concentration. On the other hand, those bulls (such as bull 49) whose semen was more nearly constant in consistency produced semen of less variability in pH. From this observation it appears that the pH of the semen is regulated by the amount of secretion from the accessory sex glands. An examination of tables 1 and 2 clearly shows, however, that concentration of spermatozoa in the semen cannot be predicted accurately from the pH.

The volume of the semen and concentration of spermatozoa were both subject to variation. A comparison of these two values in tables 1 and 2 shows that they were not correlated significantly. A small sample of semen was just as likely to contain a low concentration of spermatozoa as a high one and vice versa.

The comparison of fertility of various samples of semen from the same bull with their characteristics would have been valuable to indicate the properties of semen most indicative of its fertility. Unfortunately, not

enough cows were bred to any one bull to make such a study of significance. Hence, the breeding records and semen examinations of different bulls were compared in order to find the properties of semen most nearly correlated with fertility. These comparisons are presented in table 3. The bulls are listed in the order of their breeding efficiency. Bull 56 served only two cows, hence his apparently perfect breeding record is not comparable with the records of the other bulls which each included from 12 to 52 services per bull.

Since the percentage of abnormal spermatozoa has been presented as an index of a bull's fertility, it is of interest to study first this property of the semen of the various bulls. The breeding results with bulls in this herd have been contrary to previous ideas in that two bulls (8 and 50) which produced a large number of abnormal spermatozoa have been highly efficient while two bulls (55 and 53) which produced few abnormal spermatozoa have been of poor breeding efficiency. On the other hand, one bull (52) which produced a very high concentration of abnormal spermatozoa most of which were coiled tailed, was of very low fertility. The average percentage of abnormal spermatozoa for semen of bull 8 (35.8) is abnormally high. During the first few collections from this bull he was in poor condition and had recently been shipped a long distance. These collections of semen, although fertile, were very high in abnormal spermatozoa, above 40 per cent. Most of his later semen was below 30 per cent abnormal spermatozoa. No distinction was observed as to the type of abnormality most often associated with low fertility. Coiled tails were predominant in the semen of bulls of both good and poor fertility. A large percentage of pyriform heads was observed in semen of bulls 8, 50, and 47, all of good fertility; so this type of abnormality is evidently of no more consequence in causing infertility than are coiled tailed spermatozoa. None of the bulls produced a very large percentage of tailless spermatozoa, though all produced some. Abnormalities of the middle-pieces, undeveloped spermatozoa, and other abnormal forms included under the heading "other abnormals" were rare and probably of little significance.

The average volume, concentration, and pH of the semen did not show significant differences in those properties of the semen from these bulls of poor versus good breeding efficiency. None of the bulls was considered abnormal in any of those characteristics, however. The variation in average volume of the ejaculates among the different bulls was closely related to the relative size of the bulls within their breed.

The average initial motility ratings varied among the bulls. Bull 52 consistently produced semen which was low in initial motility and this bull was of poor fertility. Bulls 55 and 53, also of poor fertility, however, produced semen which was consistently of good initial motility. Hence, good initial motility in itself is not an accurate criterion of fertility of the bull.

TABLE 3

Comparison of the breeding records and examinations of the semen of ten bulls used in the same herd

Bull No.	No. of samples	Services per conception	Average results of semen examinations									
			Volume	Concentration	Initial pH	Initial motility rating	Maintenance of 2 motility	Abnormal spermatozoa per 1000				
								Total	Tail-less	Coiled tails	Pyri-form	Other abnormals
			cc.	*1000/cu.mm.*			*hours*					
56	2	1.00	1.85	748	6.60	4.50	36.0	81	36	17	18	10
50	41	1.21	4.56	684	6.50	3.90	74.7	236	38	60	128	10
48	34	1.29	3.98	1218	6.43	3.94	32.7	139	60	73	3	3
54	36	1.31	3.59	812	6.48	3.72	52.4	157	28	110	12	7
8	17	1.33	1.64	943	6.55	4.24	44.8	358	44	146	164	4
49	28	1.38	4.46	1060	6.25	4.75	118.4	116	25	85	3	3
47	23	1.46	6.02	723	6.38	4.14	41.4	170	41	53	70	6
55	32	2.44	4.78	947	6.41	4.29	21.3	91	30	21	35	5
52	18	3.33	2.79	1738	6.53	1.78	1.0	597	46	549	0	2
53	25	3.67	5.10	862	6.47	4.46	20.7	131	37	70	17	7

The average initial motility in semen from bulls 50, 48, and 54 was not as high as the average for the other bulls of good breeding efficiency. These were the bulls which most often gave a small, thin first ejaculate of low motility but which gave second ejaculates of excellent quality.

The property of the semen which was most significantly correlated with the breeding efficiency of the bulls was the average duration of good motility in the stored semen. This was measured by the time a motility rating of 2 (above 50 per cent of spermatozoa in motion) or better was maintained. It appears significant that although other properties of the semen of poor breeding bulls 55 and 53, did not differ from those of semen from good breeding bulls, the average duration of good motility for these poor breeding bulls was distinctly below that for the bulls of good breeding efficiency. It appears from these results that semen from bulls of good fertility should maintain a high percentage of vigorous motility for at least 30 hours in storage undiluted at 40° Fahrenheit. Those bulls whose semen maintained good motility an average of less than 24 hours were of poor fertility. It also appears that the maintenance of 2 motility in stored semen above 36 hours did not enhance the breeding efficiency. Most of the semen was used for insemination immediately after collection, however; and for purposes requiring storage periods the more viable semen might have shown to greater advantage.

DISCUSSION

This study has again demonstrated the fact that ejaculates of dairy bulls are subject to wide variations in characteristics. These variations occur among separate ejaculates of the same bull as well as among the ejaculates of different bulls.

The cause of the morphological variations among different bulls has been shown by Lagerlöf (10) to be due to genitalian defects, especially defects of the testicles. Morphological variations in the same bull's semen from day to day could hardly be explained in such manner for a healthy bull. The most reasonable explanation seems to be that abnormal spermatozoa are to be found always in bull semen as the result of some normal physiological process, possibly the aging or deterioration of spermatozoa or spermatogonia, and that some times a larger concentration of such abnormal spermatozoa will be in an ejaculate than at other times. The physiological limits of production of such abnormal spermatozoa must be dependent on the individuality of the bull. The data show that for some bulls good fertility is maintained in spite of concentrations of abnormal spermatozoa as high as 30 per cent. When this value exceeds 30 per cent and approaches 50 per cent, as with bull 52, some pathological condition must be present and the breeding efficiency of the bull will be poor. A low percentage of abnormal spermatozoa, however, does not insure good fertility.

The property of semen most nearly correlated with fertility of the bull was the time of survival of vigorous motility under storage conditions. This relationship is to be reasonably expected because time is consumed in the journey of the spermatozoa to the Fallopian tubes and also in release of the ovum. The spermatozoa which will be alive and moving vigorously after such time will have a better chance of fertilizing the ovum than will the weaker ones. The low fertility of semen which is very high in percentage of abnormal spermatozoa can be explained on this basis, too, since it is also often of poor viability. Semen of some bulls which produced few abnormal spermatozoa was also of poor viability; so factors other than morphology must be responsible for the duration of motility of the spermatozoa and thus for their fertility.

The volume, concentration, and pH of the semen within the limits here presented were not correlated with fertility. Evidently, bulls of low fertility may produce semen apparently normal in these properties.

Although initial motility of the semen of the different bulls was not correlated with their fertility, except in samples with very poor initial motility which were of poor fertility, in separate ejaculates of the same bull there was a rough relationship between initial motility and time of survival with good motility. From these facts it appears that the simplest accurate method of determining fertility of any one sample of semen is to 1, assure the fertility of the bull by determining average survival time with good motility (using five or more collections secured over two weeks' time) and 2, assure the viability of any one sample of semen from a *fertile* bull by rating motility immediately after collection. This work thus confirms the observation of Davis (4) that initial motility is the best evidence of viability of semen from a fertile bull. The variation in viability of semen of excellent initial motility is quite wide, however; so from the methods here presented it appears that storage of semen from even the most fertile bulls for periods above two or three days will seldom meet with success, and then only as a matter of chance.

SUMMARY

1. A study of the semen of ten bulls used in the University of Missouri dairy herd has been made and the semen properties compared with the bulls' breeding records.

2. The property of the semen most nearly correlated with fertility was the time of survival with vigorous motility in semen stored at 40° Fahrenheit.

3. Among the different bulls, other properties of the semen such as pH, concentration, volume, percentage of abnormal spermatozoa, and initial motility were not correlated with the bull's fertility or time of survival with good motility except in the case of one bull of poor fertility which produced semen very high in abnormalities and very low in initial motility.

4. Among separate ejaculates from the same bull initial motility was roughly correlated with viability in storage.

5. Wide variations in all properties of the semen were observed, among ejaculates of different bulls as well as among ejaculates of the same bull.

6. It is proposed that a bull's fertility should be rated from examinations of at least five semen samples collected over a period of at least two weeks, and that the suitability for use or storage of a sample of semen from a fertile bull can best be determined at time of collection by examination for motility.

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ADEQUACY OF "HOME-GROWN" RATIONS IN PROTEIN AND MINERAL MATTER FOR GROWTH IN DAIRY HEIFERS*

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The desire for economy in raising dairy cattle replacement stock, as well as in producing milk, has led to an interest in the use of "home-grown" rations. Protein supplements are the chief feeds needed on the average farm in the midwest. By the greater use of leguminous crops for the production of protein-rich hays, silages, and concentrates, the purchase of supplemental feeds may be largely avoided. Contemplation of such a feeding program raises the question of the adequacy of such rations from the standpoint of quality and quantity of protein supplied. Likewise, the mineral needs of the animals must be considered. Huffman[1] has reported that rations consisting of alfalfa hay, corn silage, and the farm-grown grains are inadequate in phosphorus for milk production and lead to inappetence and consequent unsatisfactory practical results. He found that the addition of bone meal corrected the phosphorus deficiency and was effective in keeping the animals on feed.

EXPERIMENTAL

An experiment to study the adequacy of the protein from limited as well as multiple sources and the mineral matter supplied by home-grown feeds was started with Holstein heifers averaging six months of age in January, 1934. Six lots of three animals each were started on the following rations:

Lot	*Roughages*	*Concentrates*	
1	Timothy hay	Corn	500
	Corn silage	Oats	500
		Salt	10
2	Timothy hay	Corn	370
	Corn silage	Oats	370
		Gluten meal	260
		Salt	10
3	Timothy hay	Corn	266
	Corn silage	Oats	266
		Bran	234
		Linseed meal	117
		Gluten meal	117
		Salt	10

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1 Tech. Bulletin No. 134, Mich. A.E.S., "Phosphorus Requirement of Dairy Cattle when Alfalfa Furnishes the Principal Source of Protein." C. F. Huffman, C. W. Duncan, C. S. Robinson, and L. W. Lamb.

4	Alfalfa hay Corn silage	Corn Oats Salt	500 500 10
5	Alfalfa hay Corn silage	Corn Oats Bran Salt	600 200 200 10
6	Alfalfa hay Corn silage	Corn Oats Bone meal Salt	500 500 18 10

The animals in all lots were fed according to the same plan of allowing hay, silage, and grain in the ratio of 1 : 2 : 1, respectively. Thus a heifer that was fed 5 pounds of hay per day also received 10 pounds of corn silage and 5 pounds of the concentrate mixture. This plan was followed throughout with the exception of lot 3 where it was necessary, due to the bulkiness of the mixture, to increase the allowance of concentrates 8 per cent by weight to give the same nutrient intake as for the other lots.

The nutritive ratio of the total ration was 1 : 10.7 for lot 1 and between 1 : 6.5 and 1 : 6.7 for the other lots.

RESULTS

Lot 1 received a ration similar to that on which too many dairy animals are forced to get along, at least for short periods of time, and which is known to be lower in protein than is considered advisable to feed. This ration consisted of timothy hay, corn silage, corn and oats. During the growing period of approximately 18 months (532 days) after the start of the experiment, these heifers gained from an average weight of 498 pounds to a weight of 1169 pounds, or an average daily gain for each heifer of 1.26 pounds.

Lot 2 received the same roughages as lot 1 but the concentrate portion of the ration was improved by the addition of sufficient corn gluten meal to bring the level of protein intake up to an approved standard. The heifers in this group made an average daily gain of 1.41 pounds each during the 532 days of the growth period following the inception of the experiment.

Lot 3 was fed the kinds and amounts of roughage as was given to lots 1 and 2 but a greater variety of ingredients was included in the concentrate mixture, which included corn 266 parts, oats 266 parts, wheat bran 234 parts, linseed meal 117 parts and corn gluten meal 117 parts. This ration furnished the same amount of protein as was fed in lot 2 but afforded a greater variety of feeds and presumably a higher quality of protein. The gains made by the heifers during the first 18 months on the experiment did not indicate it to be superior to that given to lot 2, since the lot 3 heifers averaged 1.30 pounds gain per head daily, during the 532 days.

In the remaining lots (4, 5, 6), alfalfa hay and corn silage were fed as roughages.

Lot 4 received corn and oats in equal parts, the same as fed to lot 1. The protein supplied by the alfalfa hay enabled these heifers to gain more rapidly than those in lot 1. The average gain was 1.45 pounds per head daily during the first 518 days on experiment.

In lot 5 a phosphorus-rich feed was added to the "home-grown" feeds in the form of wheat bran. The concentrate mixture used was corn 60, oats 20, and bran 20 parts by weight. The heifers receiving this ration gained 1.41 pounds per head daily.

Lot 6 was fed the same as lot 4 except that bone meal was added to the grain mixture of corn and oats at the rate of 18 pounds per ton. These heifers gained at the average rate of 1.46 pounds per head daily during the first 18 months of the trial.

TABLE 1

Feed consumption per unit gain and average daily gains of heifers

Lot No.	Feed consumption per 100 pounds gain				Average daily gain per head
	Concentrates	Timothy hay	Alfalfa hay	Corn silage	
1	451.3	448.6	....	897.2	1.26
2	393.3	391.6	....	782.6	1.41
3	455.5	420.8	....	843.4	1.30
4	381.6	....	379.0	758.3	1.45
5	390.0	....	386.9	773.9	1.41
6	376.7	....	374.3	748.6	1.46

The gains made on the various rations were not widely different. Statistical analysis of variance[2] showed the gain of 1.26 pounds per head daily for lot 1 to be significantly lower than the gains made by the other lots. Among lots 2 to 6 the differences in rate of gain were not statistically significant.

DISCUSSION

Lot 2, with 1.41 pounds as an average daily gain per head, exactly equalled lot 5, and was not significantly behind lots 4 and 6 with gains of 1.45 and 1.46 pounds, respectively. Thus it appears that the unsupplemented corn and oats fed with alfalfa hay and corn silage (lot 4) was fully equal in its protein quality for growth to that supplied by the ration in lot 2 where the corn and oats mixture fed with the timothy hay-corn silage roughage was supplemented by the use of corn gluten meal to bring the nutritive ratio up to approved standards. It further appears that (within the limits of this experiment) the corn protein as furnished by the gluten meal to lot 2, was as efficient in supplementing the basal ration (fed to

[2] Made by Dr. C. Eisenhart, Statistician, Wisconsin Experiment Station.

lot 1) as was the protein supplied in the more complex mixture including wheat bran, linseed meal, and corn gluten meal fed to lot 3.

The use of wheat bran in the ration of lot 5, or of bone meal in the ration of lot 6 failed to show any significant advantage over the corn and oats ration of lot 4 when all three were fed with alfalfa hay and corn silage to growing heifers. No lack of appetite (anorexia) was noted with these heifers at any time in any lot.

On June 27, 1935, after approximately 18 months on experiment, blood samples were collected and the serum was analyzed for Ca and P. Calcium and phosphorus levels were normal in all lots which indicate that the level of intake was fully adequate for growth. (See table 2.)

TABLE 2

Calcium and phosphorus levels in the blood serum of heifers and the daily intake of these elements after approximately 18 months on the respective rations

Heifer No.	Serum Ca	Serum Inorganic P	Daily intake	
			Ca	P
	mg./100 cc.	*mg./100 cc.*	*grams*	*grams*
1	11.50	5.6	14.8	15.8
2	11.50	7.0	14.8	15.8
3	11.30	6.9	14.8	15.8
4	11.00	7.1	14.4	16.9
5	11.25	6.9	15.5	18.1
6	11.60	6.9	16.8	19.7
7	11.70	7.5	17.6	27.2
8	11.25	7.8	17.6	27.2
9	11.00	8.2	17.6	27.2
10	11.60	7.1	40.0	21.8
11	11.85	6.1	40.0	21.8
12	11.80	6.4	43.5	23.2
13	11.15	6.3	44.2	30.2
14	11.10	7.9	40.6	28.2
15	11.55	7.5	37.9	26.3
16	10.65	7.6	37.9	28.7
17	11.80	7.8	40.6	30.0
18	10.90	6.6	44.1	32.2

From table 1 it will be noted that those lots which showed the largest average daily gains were the most efficient in utilizing feed for gain when the total ration fed is charged against the gains made. This is very commonly observed in cases of animals fed for meat production and these data are in harmony with such observations.

SUMMARY

Simple home-grown rations proved to be adequate for growth in dairy heifers. Alfalfa hay, corn silage, and a mixture of equal parts corn and

oats produced excellent gains. The rate of growth was not improved by the addition of bone meal or wheat bran.

Timothy hay, corn silage, and corn and oats furnished too little protein for optimum growth. The simple addition of protein from corn in the form of corn gluten meal promoted as rapid growth as was obtained by using a mixture of greater variety including wheat bran, linseed meal, and corn gluten meal in addition to corn and oats.

COMMON DEFECTS OF ICE CREAM, THEIR CAUSES AND CONTROL; A REVIEW

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In the scoring of ice creams, sherbets, and ices for defects the terms only of texture and body appear to be confusing. In a considerable portion of the research work reported the terms have been used interchangeably as well as collectively under the one heading of body and texture. In the scoring of cheese, texture has been defined as the appearance of solidity; body as the substance or consistency of the solid portion. Texture, by dictionary definition, is "the disposition or manner of union of the particles or smaller constituent parts of a body or substance; fine structure; as, the texture of earthy substances or minerals; the texture of a plant or bone," while body is defined as "the physical structure" of the substance. Turnbow and Raffetto (147), apparently because of the obscurity of the two terms, classify the two types of defects under one heading. Frandsen and Markham (52) state: "There is some difference of opinion as to whether body and texture should be judged together or be considered as two separate characters. . . . Body and texture, whether considered together or separately, are of no little importance, but they are characters which are difficult to describe. In very general terms body may be said to be that quality which gives weight and substance to the product and enables it to stand up well. . . . Texture refers to the grain or to the finer qualities of the product." Larsen and White (70) mention body and texture individually and specify defects in each but do not formally define them. Fish (50) states "the texture refers to the molecular structure. . . . The body of the ice cream refers to the structure as a whole." Ambrose (2) distinguishes between the two, "Texture refers to the size, shape and arrangement of the particles or the structure of the ice cream. An ideal texture is one which is perfectly smooth and entirely free from all graininess. Body refers to the total organized substance or the mass of ice cream as a whole. It is dependent upon the texture and resistance. It may be described as light, good, heavy, or soggy." Downs (44) considers body and texture under one heading. Handy (55) describes texture as relating "to the lightness and fineness or smoothness and grain of the body, depending upon the manner in which the ingredients have been incorporated and held together. . . . Body defines weight in the product as well as the quality which gives it lightness, resistance to melting and firmness." Although listing body and texture defects under one general group, Sommer (126) defines each individually: "texture is the attribute of a substance relating to its finer structure—the size, shape and arangement of the small particles. Body is the attribute of a substance relating to the properties of

the mass as a whole—its consistency or firmness, and in the case of ice cream its melting resistance.'' In view of these opinions and for sake of convenience, body and texture in the following reviews are considered under one group of defects.

FLAVOR DEFECTS

Trout, White, Downs, Mack and Fouts (144) have compiled lists of off flavors found in ice cream samples used at the Students' National Contests in the Judging of Dairy Products for the period 1927–1938 inclusive. Inasmuch as some of the samples were selected because of specific defects, the percentage distribution probably does not apply to commercial ice cream as a whole, but the defects mentioned are representative of those found in commercial ice creams. During the period named, 80.95 per cent were criticized on the flavor, the percentage distribution being, old ingredient, 23.02; unnatural, 20.14; condensed or cooked, 17.26; lacks fine flavor, 12.95; unclean, 6.47; egg powder, 5.03; lacks flavoring, 2.87; metallic, 2.87; neutralizer, 2.87; too sweet, 2.15; high acid, 2.15; and miscellaneous, 2.15. Smith and Tracy (124) in a study of consumer preferences have shown that as far as desirable flavors are concerned women prefer heavy, well pronounced flavors.

Old ingredient, oxidized, metallic, stale, and *tallowy flavors* are terms used interchangeably to a degree by different judges. Rice (117), in one of the early references to tallowiness, referred to the catalyzing influence of copper and light on the development of this flavor. He noted its development at low as well as at high temperatures, and that products of low bacterial content were particularly susceptible to the defect. His work led him to the belief that the action was chemical rather than bacteriological; that oxygen acted more rapidly than air in causing the defect; that filling cans full and thus reducing the space occupied by air might lessen the difficulty; that the greater the copper content, the less air was required to produce the defect; that tin does not catalyze the action; and that iron may act as the catalyst but to a lesser degree than copper. As remedial measures Rice suggested leaving no space for air in cans, cleaning the vacuum pan thoroughly after use, and exposing milk the least possible to copper surfaces.

An exhaustive abstract on oxidized flavor in milk and dairy products was published by Brown and Thurston (13) in 1940. Much material bearing in general on oxidation of fats has been omitted here because of duplication and because much of it was concerned with dairy products other than ice cream.

In 1931 Dahle and Walts (30) studied the methods of preparing dried skimmilk and the effects of method of preparation on ice cream made from it. Those skimmilk powders made from condensed skimmilk generally scored slightly higher, while atmospheric roll powder, when supplying from

eight to nine per cent of the serum solids yielded an ice cream with a decided milk powder flavor. In the same year Dahle and Folkers (31, 32) reported the frequency with which tallowy flavor was encountered in fat-free strawberry ice cream; that it was found no matter what variety of commercial strawberries was used; that the flavor did not occur consistently; that sterilization of mix and berries did not prevent it; and that the flavor did not occur when spray process skimmilk powder was used but did occur when condensed milk was used. No off flavor occurred when less than 0.8 p.p.m. of copper was present or when the acid in the berries was neutralized to pH 7. Dahle, Walts, and Keith (33) found that either spray process or vacuum roller skimmilk powder could supply as much as 10 per cent of the total serum solids, and atmospheric roller process powder only 8 per cent without imparting an undesirable powder flavor. In 1933 Dahle and Folkers (34) published results of extensive work done to ascertain causes of the frequent appearance of oxidized or cardboard flavor in strawberry and pineapple ice creams. This occurred when either fresh, cold pack, or canned strawberries were used and especially when used in 10 per cent amounts. Doubling the amount retarded its appearance but heating mix, or berries, or both, to 180° F. did not prevent the flavor. Appearance of the flavor was delayed by adjusting the pH to 7. The use of skimmilk powder as serum solids seemed to prevent it while condensed milk seemed to favor its appearance generally when it contained its usual copper content of 1.3 p.p.m. Copper content in condensed milk could be kept down by frequent use of the vacuum pan and by keeping the inside surface well polished. Link, Konen, and Baumann (72) announced in 1935 that the presence in ice cream mixes of 0.28 to 1.39 p.p.m. of copper would cause oxidized flavor to appear in strawberry ice cream in 16 to 51 days, dependent on the amount used. With no added copper the defect appeared in 65 days. Since the defect appeared only during the winter season the authors suggested that rapid turnover in the summer and diet of the cows may be factors influencing development of the flavor.

Smith (125) in 1935 recommended that packaged ice cream be held in storage no longer than a month due to the development of off flavor. In the trials made by the author the flavor developed in vanilla ice cream in approximately one hundred days, but strawberry ice cream was particularly susceptible to the defect, it appearing more quickly than in vanilla, chocolate, or maple walnut. The samples used for the experiment were stored for 16 to 24 weeks at 6 to 20° F. The stale flavor began to develop in the strawberry flavored cream after one to two months in storage, in vanilla in three months, but chocolate and maple walnut were unchanged after four months. The author concluded that age of the ingredients before freezing was more important than storage period. Schricker (121) found the off flavor to develop in vanilla ice cream in four weeks; in chocolate in four to six weeks; and in strawberry in two weeks.

Tracy, Ramsey, and Ruehe (130) in 1933 found the flavors, stale, metallic, and tallowy occurring together in strawberry ice cream. They found fat oxidation to be hastened by the presence of copper salts and a minimum of berries, the latter being true by reason, presumably, of a lack of fiber. Remedies suggested were elimination of copper contamination, use of high quality dairy products, addition of at least 15 per cent of solid pack strawberries and the use of high homogenizing pressures. The same year Ross (118), working with 22 identical mixes, except for milk-solids-not-fat, was unable to find any change in degree of fat oxidation. He was unable to locate oxidizing agents as a factor, nor iron. Some flavors, he found, might wholly or partially mask the off flavor but it was found in control mixes containing no strawberries. Mixes containing condensed skimmilk developed the flavor more quickly than others, probably because of the presence of copper together with any fat oxidation occurring during manufacture. Scism (122) in 1934 held 12 samples each of chocolate, vanilla, and strawberry ice creams at $-25°$ F., and held a second similar set of samples in an ice cream cabinet. Scorings made at intervals showed that those samples held at low temperatures resisted deterioration. At either temperature chocolate stood up best, vanilla second best, with strawberry deteriorating first in flavor.

Tracy, Ramsey, and Ruehe (131, 132) in 1934 condemned copper as being the most important of the causes of oxidized flavor, and its elimination from the mix as one preventive measure to be taken. Other measures recommended were high homogenizing pressures, heating of apparatus and either soaking the strawberries in mix before use or the use of increased amounts of fruit, or the addition of increased amounts of strawberry fiber. Denseness of the berry pack was thought to be a possible factor. The authors suggested the probability that the strawberry juice contained a catalyst and the fiber a reducing agent. Addition of citric acid to the strawberry juice did not hasten oxidation. The same variety of berries acted differently depending upon the heat treatment given them during canning, and, presumably due to varying fiber content. In desirability for use in ice cream the varieties tested rated from most to least desirable were: Dunlap, Parson Beauty, Gandy, Premier, Gibson, and Aroma. Either two, three, or four parts of berries to one of sugar were recommended as desirable packs. Other fruits than strawberries found to accelerate production of oxidized flavor were oranges, lemons, and pineapple.

Dahle and Josephson (35) found a high copper content in some pineapple products and stated that it contributed to the development of oxidized flavor. They found canned pineapple on the market, however, sufficiently low in copper to be acceptable to the ice cream trade. Sorber (128), writing in 1940, claimed that contamination of several kinds of fruit with copper will intensify the reaction of copper in the production of oxidized

flavor. In 1937, Bird, Willingham, and Iverson (7) stated that the addition of strawberries did not materially increase the amount of copper in a mix; that the development of a stale flavor in ice cream was not serious but that the presence of metallic and tallowy flavors were; that heating the butterfat in the vacuum pan apparently had no effect on the flavor; and that storage of the condensed milk for a week at 32° F. had no effect. The addition of strawberries tended to delay the appearance of off flavor in the ice cream. Presence of copper accelerated the development of the flavor but the authors believe that it is the state of the copper, rather than its mere presence, which affects the flavor production. Stainless steel pans caused less off flavor, and condensed milk from stainless steel pans gave mixes of better quality than those made from dried milk.

Iverson (62), the same year, called attention to the importance of the presence of iron in the mix in the development of tallowy flavor. He stated that the higher this is the less pronounced the off flavor will be, due, he believed, to the iron helping form an antioxidative catalyst through the action of ferrous iron with a milk constituent. He found oxidases in the fruit were not factors in the flavor production. Iodine number changes paralleled oxidative changes, but Reichart-Meissl numbers and acetyl values did not, suggesting the role of the unsaturated fats. Tallowy flavors were more often encountered when whole milk, condensed, or skimmilk condensed were used than when dry milk was used. Either type of condensed milk, when made in a stainless steel vacuum pan, showed a much reduced tendency to develop the flavor. Fruits retarded the tendency to develop the tallowy flavor. Mixes containing less than 1.18 p.p.m. of copper did not develop tallowy flavor; those showing the flavor contained at least 1.8 p.p.m. of copper. If the flavor appears between these copper content mixes, the author ascribes its cause to other factors. He also emphasized state as well as presence of copper as important. In addition to the above, in another publication, Bird, Iverson, Ause, and Willingham (8) state that strawberries contain no oxidases; that fat content of the mix had no effect on rate of development of the flavor; and, that mixes from stainless steel pans did not develop the defect.

With regard to antioxidants for prevention of oxidized flavor, Dahle and Josephson (36, 37) reported in 1937 that the use of 0.5 to 0.7 per cent oat meal flour (Avenex No. 7) would prevent tallowy flavor in strawberry ice cream with 2 p.p.m. of copper present. The use of 0.7 per cent increased viscosity too greatly. The oat flour, when added in amounts of 0.5 to 2 per cent to fresh cream before freezing for storage, reduced its tendency to develop the flavor. The authors recommend 0.5 per cent, and addition to the mix with the sugar. Mueller and Mack (95), the same year, reached similar conclusions. Of several rates used they found 0.5 per cent of oat flour to be the optimum, preventing appearance of oxidized

flavor for at least four weeks. When 0.5 per cent of oat flour was used they found that gelatin should be decreased by 25 per cent. The use of as little as 0.25 per cent oat flour retarded appearance of oxidized flavor during storage but not as efficiently as 0.5 per cent. Also in the same year Musher (100) advised the use of 0.5 per cent Avenex to prevent formation of oxidized flavor in ice cream and recommended reduction of gelatin by 25 per cent when this amount of oat flour was used.

Maack and Tracy (84) reported in 1938 that using oat flour in mixes to which 3 p.p.m. of copper had been added, and in rates varying from 0.1 to 0.5 per cent, 0.5 per cent was necessary to secure sufficient retardation. This amount added to vanilla mix was sufficient to protect it against oxidized flavor for several weeks. The oat flour was helpful in retarding development of the flavor in strawberry ice cream containing 1 p.p.m. of copper but not to the degree it was helpful in vanilla mix. It imparted a slight flavor of oat meal to the mix but this was not unpleasant. The authors recommend mixing the oat flour with the sugar and adding the two together or adding it in dry form at the freezer. An attempt to make and use a water extract of the antioxidative agent was unsuccessful.

Weckel (150) pointed to origination of off flavors from three sources: 1, inferior ingredients; 2, equipment effect; and 3, storage. In referring to oxidized flavor he suggested the use of Avenex or a trypsin preparation as being effective. Burke and Newman (14) also recommended the use of oat flour to the extent of 0.5 per cent with a reduction of gelatin. They found the flour to impart a slight oat flavor.

Dahle and Nelson (38) in 1941 published results of an effort made to isolate from two well known edible antioxidants the material responsible for their antioxidative value. Search was directed particularly towards the phospholipid content and fractions made using several solvents. Isolation of such a concentrate would greatly simplify the use of cereal antioxidants in the ice cream mix. The isolation attempt was only partially successful. The phospholipid fraction and the alcohol extract exhibited greater value than the fractions secured with other solvents. Those from oat flour proved more potent than similar extracts from soya bean flour.

Condensed, powder, and *cooked flavors* result from overuse of particular concentrated products or the use of those products which have been improperly processed. Ambrose (2) found that ice creams made with 12, 14, and 18 per cent of milk solids were at first good in flavor but after two or three weeks in storage developed condensed milk or lactic flavor. These rarely developed when less than 12 per cent of milk solids were used.

Dahle, Walts, and Keith (33) in 1931 pointed out the danger of securing a milk powder taste if more than 8 per cent of atmospheric roller process skimmilk powder or greater than 10 per cent of spray process powder were used in supplying the total solids. The same year Price and Whittaker (106),

studying the use in ice cream of spray process, flake, vacuum roll, and atmospheric roller skimmilk powders found all to be satisfactory, except the last, which gave a cooked, custard-like flavor. In 1931, also, Iverson, Calder, and Chu (63) noted that ice creams made with either roller or spray process skimmilk powders scored higher in flavor after having been stored two weeks than ice creams made with condensed skimmilk. When ice creams made with low concentration plain condensed skimmilk were stored, off flavors developed more rapidly than when made from high concentration condensed skimmilk. Ice creams made from concentrated skimmilk products and unsalted butter developed off flavors more rapidly than ice creams made from sweet cream. In practically all cases the amount of unsalted butter that could be used was limited. Gibson (54) found that the use of "Pro-lac" powder in ice cream yielded a malt flavor and salty taste. Carithers and Combs (15) in studying drum dried versus spray process powder for ice cream found each equally effective in producing a fine flavor when fresh. When held six months before use drum dried powder was much inferior to spray process and imparted a strong, old, stale powder flavor to the ice cream. Iverson (64) is authority for the statement that serum solids may be a fruitful source of off flavors. They may improve flavor, but because of their own pronounced flavor, the use of more extract in the mix is required to cover a portion of the flavor of the serum solids themselves.

Tracy (133, 134) as early as 1923 checked the relative advantages and disadvantages in ice cream of superheated condensed skimmilk. The use of the superheated product resulted in a lowering of flavor score due to the presence of cooked flavor. The use of the unsuperheated milk caused no cooked, condensed flavor. The ice creams used in Tracy's tests varied considerably in fat content but very closely resembled modern ice cream except for a low sugar content of approximately 12 per cent. On storage the superheated showed a tendency to develop both old and condensed milk tastes. The unsuperheated before holding or storage had a flavor more nearly resembling that of fresh milk than did the superheated.

Lucas (73) has stated that some off flavors may be removed from ice cream ingredients, especially from the dairy products, by making the mix in the vacuum pan, and that a fresher tasting mix results due to the use of fresh milk. Reichart and Corley (107) compared fresh and frozen condensed skimmilk in ice cream. After two weeks of storage there was no appreciable difference in flavor but after seven months of storage both were criticized as having a condensed flavor and as "lacking in fine flavor." Crowe, Deane, and Winn (25) could find no difference in the flavor of mixes made with fresh and frozen plain, superheated, and sweetened condensed skimmilk. In work reported from the Kansas Experiment Station (66) finished ice cream made with superheated plain condensed milk or superheated products other than the one mentioned showed a cooked flavor.

Superheated plain condensed milk gave a slightly cooked flavor; superheated sweetened condensed milk gave a pronounced cooked flavor; and superheated mix had a still more pronounced cooked flavor. If sugar was present when the product was superheated a caramelized flavor resulted; if not, only a cooked flavor developed. On the whole, the authors concluded, superheating was detrimental to high quality.

Unnatural flavor. Washburn (148), in the first agricultural experiment station bulletin on ice cream making, stated that flavor in ice cream is due to fat content, freedom from contamination, low acidity, addition of salt, and aging of cream. Bear (4) stated that flavor is affected by quality flavoring materials, richness of cream, taints and kinds of cream, filler, and storage of products; and, that "pure vanilla yields more pleasing flavor than cheap compounds. Natural fruit flavors are better than cheaper grades." Cole (17) recommended that to control flavor defects inferior raw materials cannot be used, inferior flavors or flavor combinations must be avoided, and that storage time must be limited and the storage temperatures low to avert off flavor development. He advised the use of pure rather than imitation flavors in ice cream. Lucas and Merrill (74) in a study of vanilla flavors scored pure extract flavored ice cream over compound and imitation flavored ice cream.

Egg powder flavor, if at all pronounced, is regarded as undesirable in ice cream because it is offensive to an appreciable number of people. Martin and Caulfield (87) have stated that use of 0.5 per cent of egg yolk did not affect flavor of the mix. They advised that egg yolk flavor is objectionable to some people and that it is more noticeable in low than in high solids mixes. Mueller and Button (96) in 1929 asserted that addition of 1 per cent of dry egg yolk to ice cream mix did not injure flavor but that 1 per cent of dry whole egg did. Addition of 1 per cent of dried egg albumin injured flavor by imparting an albumin aftertaste. The addition of either dried yolk or whole egg caused more noticeable improvement in low than in high solids mixes.

Acid flavor may have been more frequently encountered a few years ago because of a belief then that acidity in ice cream should be standardized. Certainly neutralization of partially soured mixes had in some cases been resorted to. Fisher (49) in 1923 wrote that mixes containing 0.19 per cent acid homogenized at 3000 pounds pressure yielded ice cream with fine flavor, but mixes testing 0.25 to 0.3 acidity homogenized at 2500 pounds had a good flavor but lacked freshness. Masurovsky's (89) conclusions were similar: that mixes containing 0.295 per cent acid possessed, when frozen, a disagreeable lactic acid flavor that persisted even after being flavored with caramel. Tracy (135) found that increasing acidity above 0.2 per cent tended towards flavor injury.

Flavors due to sweeteners may or may not be considered as defects.

Davis (40) in 1916 observed that decreasing sugar below normal produced an unsatisfactory flavor; while raising it above normal reduced demand because of its being too sweet to suit the public taste. In 1919, in the first article on ice cream appearing in the JOURNAL OF DAIRY SCIENCE, Frandsen, Rovner, and Luithley (53) stated that corn syrup as a sweetening agent affects the flavor of ice cream favorably when used in proper amounts, but used in excessive degree it imparts a disagreeable glucose flavor. The authors recommended 70 per cent of sucrose to 30 per cent of corn syrup. Corn sugar used alone gave a flat unpalatable flavor, or when used to replace over 50 per cent of the required sucrose. Thirty per cent substitution did not improve flavor. The use of a mixture made by inverting 1 pound of corn syrup and 2 pounds of cane sugar with $\frac{3}{4}$ pound of water and 1 gram of tartaric acid yielded a syrupy flavor. When used alone, in quantity sufficient to impart desired sweetening value (6.25 pounds per batch), this syrup caused an objectionable flavor, but when 1.25 pounds of sucrose were used with 4.5 pounds of the syrup the resulting flavor was acceptable. Ayers, Johnson, and Williams (3) in 1918 wrote that syrups made from grains contributed a grain flavor to ice creams in which they were used to replace 10 per cent of the sugar normally employed. Some grades of corn sugar on the market at that time produced a bitter flavor.

Williams and Campbell (152) in 1922 found in a consumer preference test that of 443 people a majority preferred ice cream containing 19 per cent of sugar and over 90 per cent preferred ice cream with 16 or higher per cent sugar. Reid (108) in 1924 stated that ice cream containing 12 per cent of sugar was superior in flavor. Lucas, Matsui, and Mook (75), in scoring ice creams containing varying amounts of sugar, considered 15 per cent the optimum, and 17 per cent as being a trifle too much. The inversion of sucrose was studied by Ruehe (119) in 1919. He found that substitution of corn sugar for 20 to 50 per cent of invert sugar was practicable, but that glucose was less satisfactory because of being less sweet and because of flavor. In 1930, Smallfield (123) stated that lower grades than light amber of honey lent too strong a flavor to ices and sherbets. He found it undesirable to use honey in whole milk sherbets on account of its not blending well but found it satisfactory when mix was used to supply the dairy product in them. Tracy *et al.* (136, 131) stated that corn sugar had little effect on flavor and that it had no tendency to prevent or delay the development of tallowy flavor. Corbett and Tracy (22) in 1936 corroborated their earlier statements on corn sugar by stating that it had no detrimental effect on flavor. Corbett and Tracy (23) and Tracy (137) in 1939 stated that consumer preference tests indicated that dextrose was 0.83 as sweet as sucrose and that consumers thought dextrose sweetened ice cream richer when one fourth of the sucrose per pound per batch was replaced with 1.43 pounds of dextrose. Cooley,

Reid, and Hall (21) in 1940 replaced one fourth of the sucrose in ice cream mixes with equal amounts of dextrose. Four hundred consumers found the resulting ice cream as desirable in flavor and in some instances more so than when sucrose only was used as the sweetener. Dahlberg and Penczek (27) found a new type of liquid corn syrup gave to ice cream a fresher, fuller flavor than sucrose. Dry corn sugar gave a slight flat flavor due to low sweetening value. Tracy (138), working with "Sweetose," a high conversion corn syrup, used it to replace 33 to 50 per cent of the sucrose in ices and sherbets. He found it to improve some fruit flavors, especially pineapple. When used to replace 25 to 33⅓ per cent of the sucrose it had no effect on flavor.

Flavor defects in chocolate ice cream. Caulfield (16) in 1930 studied the effect of several commercial chocolate syrups for ice cream and found most of them very high in sugar. Adjustment of composition of the mix to allow for the composition of the syrups corrected the difficulty. The syrups produced extremely variable flavors which were in no case as desirable as those obtained from the use of high grade chocolate liquor or cocoa. Tracy, Ruehe, and Tuckey (139) in 1930, in studying chocolate liquor versus cocoa for ice cream, found that increased serum solids and fat produced a more pleasing flavor. By increasing the sugar content to 17 per cent a bitter flavor was avoided. Reid and Painter (109) found that when using in excess of 17 per cent of sugar in chocolate ice cream the product lacked chocolate flavor and that the use of chocolate flavoring produced a true chocolate flavor.

Maybee (90) in 1939 stated that flavor strength in cocoas and chocolates depends on the fineness of grinding: the more finely the product is ground, the stronger the flavor. Meeker (91) stated in 1940 that a portion of the flavor of cocoa beans lies in the cocoa fat. This conclusion was drawn from results of many tests made by a large chocolate manufacturing firm in which, using the same blend of beans for each, ice creams made with chocolate liquor were better than ice creams made with cocoa or a mixture of the two. By calculation, the same amount of non-fatty constituents was used, thus introducing the same amount of non-fatty flavor plus any carried in the additional cocoa fat. It was claimed that chocolate liquor flavored ice cream possessed a truer chocolate flavor. Lucas and Gould (76) in an extensive series of consumer tests of preference for individual varieties of cocoa beans ground into liquors found the majority of consumers wanted chocolate of pronounced flavor. South American bean liquors were preferred. Ash, pH, and cocoa fat content of the beans used had no influence on flavor.

Miscellaneous flavor defects. Bishop and Murphy (9), in early studies of homogenization, stated that the process produced a richer flavor in ice cream. Ambrose (2) observed in 1923 that richness of flavor was increased by increasing the per cent of fat provided the milk-solids-not-fat were

reasonably high. If these were as low as 6 per cent an increase of fat from 8 to 14 per cent did not affect richness. In this respect fat and milk-solids-not-fat were supplementary. Increasing fat beyond 14 per cent did not enhance richness of flavor. Lucas, Matsui, and Mook (75) found an increase of 2 per cent of fat above 10 and up to 14 per cent of fat increased flavor score three points. Eight per cent fat ice creams were described as thin and watery. From 12 to 14 per cent butter fat was regarded as ideal.

Tracy (140), in 1930, seeking to locate a bitter, woody surface taint appearing after two to three days storage in whipped cream used on fancy goods, found it to be an absorbed flavor coming from wood surfaces exposed in ante and hardening rooms. Yellow pine was found to be especially faulty but ash was also contributory. Woods free of odor when dry were found to produce it when wet. Sealing ice cream containers as nearly air tight as possible was recommended. Fabricius (48) is authority for the statement that agar and gum, used at times to control overrun, may impart undesirable flavor to ice cream. Baer (4) in 1916 stated that old, tainted, and sour cream carry their respective flavors into the finished ice cream and that excessive stabilizer can usually be tasted. Williams (153) in 1917 wrote that in some cases gelatin was found to give ice cream an undesirable taste and in some cases it tended to blend the fruit and cream flavors. When used in excessive amounts it subdued a portion of the fruit flavor. Tuckey (146) found that low grade gums gave an off flavor to ices and sherbets. This flavor might be described as "medicinal" and bitter. Plastic cream, according to Foskett and Mack (51), in trials made by them, did not give as good flavor to ice cream as sweet or frozen cream, but was better than butter as a source of fat. Joslyn and Cole (65) in 1938 recommended against the use of fruits high in tannic acid. They stated, also, that high fat mixes tended to mask the flavor of fruit in fruit ice creams.

Reid (110, 111) has said that serving temperatures affect flavor of ice cream. Vanilla and strawberry were best served at 16° F. and chocolate at 10° F. Served at 16° F. the flavor of the chocolate was too pronounced. All flavors served at lower temperatures lacked flavor and were too cold to be most palatable. The later work recommended as serving temperatures 10° F. for ice creams of mild flavor, and 16° to 18° F. for those of more pronounced flavor.

BODY AND TEXTURE DEFECTS

Trout, White, Downs, Mack, and Fouts (145) compiled criticisms and their incidence in scoring ice creams in the National Dairy Products Judging Contest and found that 74 per cent of the 104 samples were criticized for body and texture. The most frequent criticism was that for coarseness, 22.6 per cent. Incidence of other defects in percentage were: curdy whey off, 18; weak body, 15.8; crumbly, 9.6; does not melt, 8.9; icy, 8.2; soggy, 8.2; fluffy, 6.2; and buttery, 2.1. Few perfect scores were given and the average cut in body and texture was two points.

Cole (18) stated in 1932 that texture and crystal structure were synonymous. He preferred to determine structure microscopically, using a rotary microtome to cut a section, mounting it in a fixative on a glass slide, magnifying 100 times, and reflecting light up through the slide by means of a mirror. He found that rate of freezing affects size of ice crystals, that proper freezing and storage may correct many texture defects, and that smoothness of texture as determined by size of ice crystals does not correlate with size or volume of air cells. Cole and Boulware (19) reported in 1940 a study, made by means of a dilatometer and organoleptic tests, of ice crystal formation in ice cream. They state that texture defined by examination microscopically would depend on ice crystal size and structure, and by organoleptic tests would depend on size of ice crystals, temperature of sample, and constituents supplying lubricant-like properties. High-fat mixes were smooth but did not necessarily contain smaller ice crystals. Composition remaining the same, ice crystal size would influence smoothness. Butterfat often masked coarseness, as when high fat ice cream is frozen in a batch freezer where large ice crystals are formed. The authors found milk-solids-not-fat retarded growth of ice crystals more than fat and that, if ice crystal size alone were responsible for smoothness, milk-solids-not-fat were more important than fat. They suggested that this may be due to the affinity of the hydrophylic colloids of milk for water and its effect on ice crystal formation. Increase of fat and milk solids smoothed texture and reduced size of ice crystals. When fat content was held constant, a lowering of skimmilk solids increased ice crystal size with consequent coarseness. This effect was greater when fat was varied. Skimmilk solids retarded freezing more than did fat. Fat content had a greater effect upon texture, judged organoleptically, than a reduction of ice crystal size determined microscopically or a retardation of ice crystal growth measured by the dilatometer. With composition held fairly constant, smoothness of ice cream, determined by organoleptic tests, correlated closely with ice crystal size except where the ratio of fat to milk-solids-not-fat was very high or very low. Smith and Tracy (124) in 1938 published results of a consumer preference test. One hundred seventy-nine questionnaires were answered. The preference was for smooth texture and body, medium fat content, high serum solids, and medium to high sugar. Women preferred a heavy body ice cream and seemed to prefer the use of a stabilizer.

Coarse, granular, icy defects in ice cream have received much attention from students of ice cream making. Penny (105) in 1892 wrote that when cream from the Cooley and centrifugal systems of creaming were used in making ice cream the former proved slightly better in insuring smooth texture. He believed the treatment cream received after separation, such as age, temperature, and acidity, was more important than separation. Alex-

ander (1) in 1909 found coarseness developed in ice cream without gelatin and that gelatin hindered coagulation of casein much the same as it hinders coagulation of silver nitrate before precipitation with hydrochloric acid. Washburn (148) in 1910 ascribed good body as being due to sufficient fat, fillers, and thoroughly cooled cream; and fine texture to richness of cream, aged cream, proper freezing, and gelatinous fillers. He asserted that a filler is used to give body, and a binder to prevent coarseness when the ice cream is held. Mortensen (92) in 1911 stated that iciness and coarseness in ice cream were caused by improper packing, holding too long, absence of filler, too thin cream, and packing while too soft. Mortensen (93) in 1915 wrote that freezing the mix too soon after pasteurization gave a coarse body.

The same year, Brainerd (12) stated that smoothness is closely related to the time an ice cream will retain its texture and depends on the amount and fineness of division, within limits, of the solids present and the size and distribution of the ice crystals. Ice crystal size was regulated by the number and nearness of finely divided constituents that, interfering with ice crystal formation, held the size to a minimum. Colloidal solids, other than fat and well emulsified fat, both in a very fine state of division were best for ice cream, hence the value of homogenization. Fineness of division of the solids tended to prevent separation of the solids from the liquid phase and bore a strong correlation to the keeping qualities of the mix. This was true up to the point at which the solids merged into true solution. Bishop and Murphy (9) in 1913 advised that ice cream made from homogenized cream possessed a much improved body and smoother texture. Baer (5) the next year published results in which he stated that too rapid freezing caused soggy, coarse grained ice cream and too slow freezing caused an open, foamy texture. Aged mixes gave firmer, smoother ice creams. Raw cream produced a smoother texture than pasteurized cream but homogenization and aging corrected this deficiency in pasteurized cream. Solids, in addition to those present in cream, had to be added to give desired body. Two years later Bear (4) published results secured from 600 freezing tests in which he averred that body (general firmness) and texture (smoothness) was influenced by age, richness, and kind of cream used, serum solids content, and kind and amount of filler. Aging of cream for 24 to 48 hours at low temperature was recommended for production of smoothness. Richness of the creams used ranged from 8 to 30 per cent, but if below 18 per cent the ice cream was weak in body and coarse in texture. The thinner the cream the more filler was required. Freezing too rapidly caused coarse texture and weak body; too slowly caused lack of smoothness because the mix was not whipped properly. Excessive over-run resulted in poor body and texture, the latter being open, light, and foamy.

Mortensen (94) stated in 1918 that where raw cream was used for ice cream the texture of that made from fresh cream was slightly inferior to

that made from aged cream. The body of that made from fresh cream was coarse and weak; from 24-hour-old cream, fairly satisfactory; and from 48-hour-old cream, a trifle light. When homogenized, fresh and 24-hour-old cream produced very good body. Baer (6), the same year, stated the addition of 2 per cent of skimmilk powder to emulsified cream retarded crystallization of the ice cream. During emulsification with steam, water to the extent of 10 to 15 per cent was added to the mix, due to condensation. This resulted in coarseness of texture.

Williams (153) stated in 1917 that gelatin, repeatedly heated, lost some gelatinizing power and caused weak body. He suggested that it be used the same day prepared and that it be heated to 160° F. before addition to the mix. When ice cream, made with and without gelatin, was stored 24 hours the latter became unsalable because of coarseness and granulation. When held for six months the gelatin-made ice cream was much preferable both in flavor and body. In 1923 the statement was made in a report from the New York Experiment Station (102) that air cells in ice cream retain their size and shape due at least partially to gelatin and homogenization. These investigators found that fat had little effect in ice cream although it retarded growth in size of ice crystals. Air cells that were small and uniform in size resisted better than large cells the pressure of freezing, thereby increasing smoothness of texture.

Fisher (49) found mixes containing 0.19 acid homogenized at 3000 pounds pressure, and mixes containing 0.25 to 0.3 acid, homogenized at 2500 pounds, produced ice cream of fine body and texture. He believed the theory of the necessity of acidity for smoothness and overrun incorporation to be false.

Ambrose (2) in 1923 made mixes containing 8, 10, 12, and 14 per cent fat and 6, 10, 12, 14, and 16 per cent milk-solids-not-fat, respectively, with 12 per cent sugar and 0.5 per cent gelatin. When low milk-solids-not-fat and milk solids were used texture was coarse and body light. When high the body of the ice cream was soggy. The effect of total milk solids seemed to depend upon whether they were high in fat or solids-not-fat. With milk-solids-not-fat at the proper level the addition of fat improved flavor and body and smoothed the texture. The author added, however, that there is an optimum and upper limit for fat content. Low total milk solids, 12 to 20 per cent, caused coarse texture and light body; high, 28 to 32 per cent, caused heavy, soggy body. Per cent of these was not so important as kind. Six per cent of milk-solids-not-fat caused very coarse texture and light, snowy body, due to small internal resistance to churning during freezing, while 18 per cent caused velvety texture but very heavy, soggy body. Good results lay between these extremes. Between fat and milk-solids-not-fat, the latter was the more important in control of body and texture. For good commercial ice cream the author recommended 8 to 14 per cent of butterfat

and 10 to 12 per cent of milk-solids-not-fat. Tracy (135) in 1924 asserted that, overrun being held at a constant level, an increase in fat greatly improved texture of ice cream.

In 1928 De Pew (42) stated that viscosity variations in the mix, due to varying homogenizing pressures, caused great variation in smoothness of ice cream, and that such viscosity necessary for ease in handling and the securing of optimum overrun was best. More than that was deemed unnecessary. Reid and Skinner (112) in 1929 homogenized commercial ice cream mixes at varying pressures using single and double stage valves. Except where excessive pressures were used, aging and increased pressure improved both smoothness and closeness of texture. Reid and Garrison (113) found smoothness of ice cream increased as the temperature at which it was frozen decreased. Length of freezing time was of minor importance. Mixes of higher milk-solids-not-fat required lower homogenizing pressures than those of lower content.

Hening (56) in 1930 reported that aging the mix longer than two to four hours had no effect in improving texture except in a very few cases. Lucas (77) found a year later in using a modern freezer equipped with sufficient beaters that aging had no apparent effect on body and texture although it increased speed and amount of air incorporation. In 1933 Mueller and Frandsen (97) reported that aging the mix at 68° F. for four hours increased the efficiency of gelatin in ice cream, whether or not the mix was aged later at low temperatures. This resulted in smoother texture.

Munkwitz and Meade (99) in 1931 reported that dull freezer blades lessened freezer efficiency and caused coarse ice cream, and, if very dull, caused an increase in freezing time of 100 per cent. The authors drew ice cream at 80 per cent overrun, hardened it for 48 hours, broke it, hardened the surface with dry ice, and photographed it. Coarseness was revealed when the blades were only slightly dull. They advised that running the freezer when empty or with wash water or for freezing ices will dull the blades.

Reid and Hales (114) have stated that crystalline structure of ice cream was affected by composition of the mix and that as these mix constituents were increased texture changed from coarseness to smoothness. Fine texture, as seen under the microscope, was associated with the appearance of uniformly dispersed small, angular shaped ice crystals and jagged air cell boundaries.

Horrall (61) in 1933 listed several factors causing coarse texture. One was the drawing of ice cream in a soft or medium soft state. Heat shocking ice cream at increasingly high temperatures caused increasing coarseness when rehardened. Low storage temperatures smoothed the texture. Ice creams processed at 165° to 180° F. were smoother than those processed at 150° F. Aging had little effect on texture. High gelatin ice creams homog-

enized at low pressure were not as coarse and icy as those with low gelatin content homogenized at high pressure. Increase of gelatin increased smoothness. When gelatin was varied from none to 0.4 per cent the unfrozen serum drained to the bottom of the container making that portion quite icy.

Hening (57) found that when gelatin was not used in the mix, two stage homogenization at 2000–500, 1200–400, and 900–300 pounds pressure, as well as centrifugal homogenization caused coarse ice cream. Addition of gelatin restored the effectiveness of either type of homogenization. Higher pressures than those indicated also increased the effectiveness. Bradley and Dahle (11) in 1933 stated texture is affected by ice crystal size and this by speed of freezing. Consequently, ice creams drawn at the same temperatures from the freezer may vary in the amounts of ice present. Speed of hardening affected texture similarly: the more rapid the rate of hardening the smaller the ice crystal size and the smoother the ice cream. Ice creams from a batch freezer were coarser than from a continuous freezer due to slower freezing with accompanying larger ice crystal size. Texture of batch frozen ice cream was affected by fat content. This was not true of continuous frozen ice cream. Increase in butterfat increased time required for hardening, in turn increasing coarseness. As remedies, the authors recommended air circulation in the hardening room, drawing ice cream at low temperatures, and at low overrun. Dahle (39) stated in 1936 that for improving texture butterfat proved to be more important than serum solids because it offered mechanical obstruction to the formation of ice crystals. Homogenization, by breaking up fat groups, increased the obstruction. Other observations were previously mentioned (11).

Levowitz (71) in 1939 noted that lowering the overrun on ice creams frozen in continuous freezers caused the ice cream to be drawn in a moist condition and that, during hardening, areas froze into lumps of ice. The machine produced a dry surfaced ice cream down to 90 per cent overrun which became increasingly moist to 60 per cent overrun at which lumps of ice began to appear. At 50 per cent overrun the lumps were as large as puffed rice grains. Their appearance was delayed by increasing the total solids of the mix. Erb (46) in 1940 called attention to the drawing of ice creams from the freezer at high temperatures and hardening slowly as being causes of coarseness. An ice cream drawn at a low temperature and hardened slowly was, on the average, equal to one drawn at a high temperature and hardened rapidly. Ice creams drawn from a continuous freezer at the same temperature as from a batch freezer were smoother due to finer incorporation of air. Martin, Nelson, and Caulfield (88) collected 318 commercial samples of ice cream. A positive phosphatase test appeared to be correlated with flavor, texture, body, and color in the samples. On the average an increase in butterfat was accompanied by an increase in score (total score minus bacterial score). Samples weighing 4.50 to 5.49 pounds per

gallon, with few exceptions, scored highest in body and texture. These weights correspond to 102 and 66 per cent overrun, respectively. Over and under these amounts of overrun the trend of the body and texture scores was downward. Specific defects in the samples were not given by the authors.

Frandsen, Rovner, and Luithly (53) found corn syrup improved body and texture when it replaced 30 per cent of the sugar ordinarily used. Davis (40) found that decreasing sugar below normal caused unsatisfactory texture while increasing it greatly improved texture. Ruehe (119) stated in 1919 that glucose as a sweetener caused a coarse grained ice cream. Lucas, Matsui, and Mook (75) found 15 to 17 per cent of sugar strengthened body of ice cream. Tracy (136) claimed in 1936 that corn sugar caused smoother texture and softer body in ice creams. Corbett and Tracy (22) the same year reported that dextrose caused a heavier bodied ice cream than sucrose when frozen in a continuous freezer but that the body was not objectionable. Corbett and Tracy (23) in 1939 stated that replacement of 25 per cent of the sucrose in ice cream with dextrose had little, if any, effect on body and texture. Dahlberg and Penczek (27) found a new type of liquid corn syrup improved body and texture slightly, and the syrup, new type dry corn sugar, and corn sugar eliminated development of sucrose crystallization. Tracy (138) found that when "Sweetose," a high conversion corn syrup, was used to replace 25 to 33⅓ per cent of the sucrose in ice cream mixes improved body resulted.

Tracy (133, 134) in 1923 stated that superheated condensed milk caused ice creams containing it to be heavier in body and smoother in texture. In these respects it was deemed superior, but inferior in flavor. The Kansas Experiment Station (66) announced in 1930 that ice creams made with superheated condensed milk and homogenized without gelatin were coarse and rough. On the whole, it was stated, superheating was detrimental to high quality. Lucas and Jensen (78) found that ice creams with 50 per cent or more of the fat content coming from butter tended slightly towards coarseness. Aging 48 hours improved the body and texture. Reichart and Corley (107) found that frozen condensed milk in ice cream caused iciness and coarseness when the ice cream had been held two weeks in the hardening room. Under practical conditions, they state the defect would be hardly noticeable. Iverson (64) stated that serum solids, used correctly, improved body of ice cream.

Smith (125) stored quart and pint packages of vanilla, chocolate, strawberry, and maple-walnut ice creams for 16 to 24 weeks at – 6° to – 20° F. and then scored them at intervals. Body and texture were not changed by storage. Except for chocolate, ice creams stored in unlined packages developed an icy surface film in three to four months. Tracy, Ruehe, Tuckey, and Ramsey (141) fed three groups of cows identically, except that the protein

came from different sources. Mixes were made from cream from each of the three groups. Feed had no effect on whipping ability. Ice cream from each group made in the continuous freezer was smoother than that made in the batch freezer but, at low overrun, was more gummy. Excessive amounts of gelatin in mixes frozen in the continuous freezer favored gummy texture and made overrun incorporation difficult but not so much as with the batch freezer. High serum solids favored gummy texture. A 10 : 10 : 15 : 0.3 mix was much better when made in the continuous freezer than one with 12 or 16 per cent of serum solids. Maack and Tracy (84) found that oat flour improved body and texture, as did Mueller and Mack (95).

Sandiness. Bothell (10) in 1920 observed that sandiness of ice cream was due to lactose crystallization. In 1920 and 1921 Lucas (79, 80, 81, 82) reported sandiness in ice cream was due to crystallization of the lactose, that the critical serum solids content appeared to be 10 per cent, and that combinations of gums and gelatin tended to prevent it (later proved erroneous). In 1921 Zoller and Williams (155) prepared sandy ice creams and removed the crystals by centrifuging. Microscopic examination showed them to be lactose crystals of the alpha form. Solubility tests confirmed the conclusion.

In 1922, Williams (154) ascribed sandiness in ice cream as due to: 1, sandy concentrated milk; 2, excessive serum solids and therefore excessive lactose in the mix; and 3, hardening room temperature fluctuation. In 1922, also, Palmer and Dahle (104) studied lactose in solution at 0° F. At 32° F. its solubility was approximately 10.5 per cent. Starting with 15 to 17 per cent solutions it was possible to keep 12.5 to 13.5 per cent in solution, even when held 14 days at 0° F. Neither stabilizers nor acids had any effect on its solubility. Zero temperature delayed crystal growth more than temperatures of 15 to 25° F. In 1921 the Oklahoma Experiment Station (103) announced that inclusion of more than 12 per cent serum solids in the mix might result in sandy ice cream. Skimmilk powder and sweetened condensed milk were used to increase the total solids to 38 and 40 per cent without causing sandiness.

In 1922 Des Jardins (43) listed four causes of sandiness in ice cream: 1, use of products containing undissolved lactose; 2, use of products high in acidity; 3, mixes high in serum solids; and 4, heat shocking. He recommended avoidance of these conditions, and proper pasteurization and aging to dissolve any crystallized lactose. In 1923 (Ambrose (2) stated that mixes high in milk-solids-not-fat developed sandiness. The defect was frequent with milk-solids-not-fat at 14 to 18 per cent but developed in only two cases when less than 14 per cent. It appeared after a two to three weeks storage period and most frequently after three weeks.

Whittaker (151) set 12 per cent as the upper limit for milk-solids-not-fat in order to prevent sandiness. The longer the ice cream was in the freezer and the lower the temperature the sooner lactose crystallized. Rapid hard-

ening retarded crystallization and conversion of beta to alpha lactose. Conditions remaining the same, bulk ice cream developed sandiness quicker than packaged. Fluctuating temperatures in the ice cream cabinet hastened development of sandiness but mechanical shock proved only an insignificant factor. Nuclei caused more rapid crystallization. Sandiness developed much later in mixes pasteurized at 175° F. than when pasteurized at 145° F. or 160° F. Homogenization temperatures had little effect but presence of nut meats facilitated crystallization, due at least in part to their water absorptive power. Increase in fat clumping materially retarded crystallization.

Webb and Williams (149), to avoid sandiness, manufactured low lactose concentrated skimmilk for ice cream use by removing 65 per cent of the lactose without dispersing the proteins. To prepare it, 5.9 pounds of sucrose was added to 100 pounds of fresh skimmilk, the mixture warmed to and held at 63° C. for 10 minutes, evaporated in a vacuum pan to 70 per cent total solids, cooled to 25° C., held at 10° C. for 20 hours, after which the crystallized lactose was removed either by centrifuge or filter press. When used to supply 11 to 13 per cent serum solids in mixes no sandiness occurred and texture was improved. Sommer (127) stated in 1936 that, mix formulae remaining the same, serum solids in milk are higher in the winter because milk is higher in solids-not-fat in winter than in the summer. He mentioned this as a reason, as well as slow turnover, for sandiness being more frequent in winter than summer.

Gibson (54) used several milk concentrates for prevention of sandiness. "Pro-lac" powder caused a sticky, spongy body; "High Solids" above 6 per cent a dry, powdery body; spray and vacuum roll powders the same; and atmospheric roll powder a dry, rough body. Excessive serum solids caused sandiness more quickly. "High Solids" retarded the tendency. Pasteurization at 160° to 175° F. delayed sandiness no longer than pasteurization at 145° F. Homogenization had no effect. Keith, Rink, and Weaver (67) found that addition of sodium bicarbonate and sodium citrate to the mix decreased viscosity, clumping of fat globules, and titratable acidity. Calcium carbonate had the opposite effect. In the last case all samples of ice cream, including those containing nuts, developed sandiness. With sodium citrate sandiness was hastened with dry nuts and slightly hastened with nuts treated with hot simple syrup.

Erb (47) in 1931 attempted to control sandiness in ice creams containing 17 per cent of serum solids by replacing sucrose with equal parts of a mixture of 20 per cent maltose syrup, 25 per cent of dextrose, and 25 per cent of invert sugar. No variation prevented lactose crystallization, although by holding water content constant and using 12, 15, 18, and 20 per cents of sucrose with proportional decreases in fat, crystallization was retarded. In aqueous solutions of lactose the presence of no other sugar affected crystalli-

zation. In 1931 and 1933 Reid and Powell (115, 116) named three conditions responsible for sandiness: excess of serum solids, presence of nut meats, and heat shocking. Alkali and acid hastened final solubility of lactose but hastened crystallization once it had started. Grape nuts did not act in hastening sandiness as did ordinary nuts. The influence of the latter was lessened by boiling the meats before use in water, sugaring and autoclaving them, or by gelatinizing them. The first two processes were not recommended for practical use. Iverson (64) corroborated in 1937 the findings of other investigators that excessive serum solids in the mix would cause sandiness. Corbett and Tracy (23) in 1939 stated that replacement of 25 per cent of the sucrose in mixes with dextrose delayed slightly development of sandiness in high solids mixes. Dahlberg and Penczek (27) in 1940 stated that new type liquid corn syrup, new type dry corn sugar, and corn sugar had no great effect on development of sandiness.

Tracy and Corbett (142, 143) in 1939 described the preparation and use of a low lactose milk for replacing a portion of the serum solids in mixes for prevention of sandiness. They found its use not only effective in this respect but found it improved body. The authors listed several recipes for its use. Decker, Arbuckle, and Reid (41) in 1939 examined sandy material under a petrographic microscope and found alpha hydrate and beta anhydride lactose crystals with the former predominating. The former crystallized sooner than the latter. They found that in high serum solids ice cream held for 12 months at $-10°$ F. sandiness did not develop. The same ice cream held a time in a cabinet at 6° to 10° F. and subsequently at $-10°$ F. for five months showed pronounced sandiness, an illustration of the effect of varying temperatures.

Melting resistance and hardness. Stebnitz (129) stated that in melting ice cream should give up its air quite readily and change to a rich creamy liquid and that its melting behavior is influenced by amount and kind of stabilizer and the heat treatment given the mix ingredients. Holdaway and Reynolds (60) in 1916, using in one gallon of basal mix: 1, one ounce of gelatin; 2, 0.4 ounce of gelatin; 3, 0.4 ounce of cooked corn starch; 4, 0.4 ounce of gum tragacanth; and 5, control sample, found their hardness and melting resistance to be in the order named. When 8, 19, and 30 per cent cream was used in the mixes the score was the same except that 4 and 5 exchanged places. In hardness, ice creams made without filler from 30 per cent cream were firmest and from 8 and 19 per cent cream equally firm. Melting resistance increased with increase in fat content especially between 8 and 19 per cent. Those containing gelatin up to one ounce per gallon were hardest and most heat resistant. Gum tragacanth stabilized ice cream was smooth and soft. With this stabilizer an increase in fat decreased hardness and resistance to heat. Corn starch compared favorably with gelatin but caused a granular ice cream.

Manhart (86) used in ice cream mixes three grades of gelatin of poor, good, and very good quality at the rate of 0.6 per cent. The better the grade of gelatin the greater was the resistance to heat of the ice cream in which it was used. Ice creams containing a poor grade were more resistant to heat than those containing none. The correlation between gelatin and resistance was significant.

Ambrose (2) stated that increase in serum solids and fat increased resistance to melting. Tracy (133) found that the use of superheated condensed milk in ice cream increased resistance to heat. Lucas, Matsui, and Mook (75) found in 1933 that use of in excess of 15 per cent of sucrose, because of melting point lowering, caused ice creams to have poor standing up qualities.

Caulfield (16) in 1930 found the same to be true with several commercial chocolate syrups. Dahlberg and Penczek (27) noted that replacing more than 25 per cent of the sucrose with corn sugar reduced the melting point and softened the ice cream. Corbett and Tracy (22) also found this to be true.

Mueller (98) observed that when ice creams melt down slowly the melted portion has a curdled appearance. Ice creams showing this tendency had been aged at a high initial temperature. The author believed this to be an important factor in such cases. Foskett and Mack (51) have stated that plastic cream in ice cream mixes affected melt down adversely. Lucas and Jensen (79) found that butter supplying 50 per cent or more of the fat in ice cream lowered melting resistance; milk powder versus condensed skimmilk had no effect; nor did prolonged aging. Reichart and Cooley (107) noted that ice creams made from frozen condensed skimmilk melted down twice as fast as when made from fresh condensed skimmilk. Mueller and Mack (95) found oat flour to increase melting resistance. Crowe and Winn (26) and Crowe, Deane, and Winn (25) observed that ice creams made with fresh superheated condensed skimmilk melted more slowly than those made either with fresh plain condensed or sweetened condensed skimmilk, but, when these were stored from two to three months, exactly opposite results were obtained, storage, it was believed, causing the change. The differences, however, were not great. Superheated condensed skimmilk, either fresh or frozen, lent to ice cream more resistance to heat. It produced less foam on melting. Fresh sweetened condensed skimmilk produced most foam, but, when stored one month before use, plain condensed skimmilk produced most foam. Ice cream made from plain condensed skimmilk melted down smoothly while that made from stored frozen superheated skimmilk melted often with a curdy appearance, the sides frequently falling off in chunks. Ice cream from fresh sweetened condensed skimmilk always melted smoothly and evenly but if stored the melted portion often had a flaky or curdy appearance.

Shrinkage. Kohler (68, 69) ascribed shrinkage as due to six causes:

stability of the milk-solids-not-fat, especially those from condensed skimmilk, processing, freezing, hardening, transportation, and storage. He considered the first as most important and suggested its regulation by addition of calcium and sodium phosphates and citrates. High temperature pasteurization, high pressure homogenization, high speed continuous freezing, and excessively low hardening temperatures, he held, increased instability of serum solids and increased shrinkage. Shrinkage caused by high pressure homogenization, poor freezing, and faulty transportation was not severe. High overrun or type of package, paper or metal, were not causes of shrinkage. Use of low Bloom gelatin, he found, might introduce additional calcium. If used, he advised that a small amount of sodium bicarbonate be added at the start of pasteurization, followed by an equal or slightly smaller amount of disodium phosphate when the temperature reached 100 to 120° F. Lucas and Roberts (83) called attention to the probability of 10-per-cent-fat ice creams, containing less than 10 per cent serum solids, sinking in the container. Cole (20) stated that ice creams frozen at low and shipped to high altitudes may expand and under reverse conditions the opposite may happen. Shrinkage having occurred, change in pressure did not restore the volume. Under these conditions the harder the ice cream the less the shrinkage.

Soggy, sticky, dry, and crumbly body defects. Reid and Painter (109) observed in 1933 that chocolate ice cream containing in excess of 17 per cent sugar had a soggy, sticky body and texture. Egg powder in chocolate ice cream produced smooth texture. Reid (110) noted that when ice cream was served at 20° F. or higher it was soft and the body tended to be gummy and sticky. Corbett and Tracy (23) found that when ice creams containing 25 per cent dextrose were frozen in a continuous freezer the body was slightly sticky unless gelatin was reduced by one fourth. Dipping losses were slightly increased. Lowering the dipping temperature one degree did not remedy the situation. Heyl and Tracy (59) reported in 1940 that overfreezing on a continuous freezer or drawing ice cream at too low temperatures, when enough gelatin has been added to permit drawing the ice cream at higher temperatures, resulted in gummy, sticky body. They advocated the use of such quantity of gelatin as would produce good body under the conditions peculiar to the plant. Iverson (64) found excessive serum solids to cause sogginess.

Hening and Dahlberg (58) reported in 1923 that addition of lactic or citric acid or the natural development of lactic acid to 0.3 or 0.4 per cent in mixes before pasteurization and homogenization caused hard crumbly body. Neutralization of the acid from 0.3 to 0.4 per cent in mixes before pasteurization and homogenization caused hard crumbly body. Neutralization of the acid from 0.3 per cent with sodium bicarbonate gave a better flavor than when sodium or calcium hydroxides were used. Mack (85) in 1934 asserted that high solids in the mix caused excessive viscosity and

crumbly body. It was usually caused by the substitution of butter or frozen or plastic cream in part or wholly for sweet cream. Increasing sugar content to 16 or 17 per cent helped to remedy the defect. If this degree proved too sweet he found substitution of 3 to 4 per cent of sucrose with corn sugar helpful. Mixes containing 18 to 20 per cent fat were apt to produce crumbly ice cream. For these he recommended three stage homogenization: 2000, 500, and 150 pounds pressure for 18 per cent fat mix; and, 1500, 500, and 150 pounds for 20 per cent fat mix. Corbett (24) made ice cream using 3.5 to 4.5 per cent low lactose serum solids with 9.5 to 8.5 per cent ordinary serum solids and found the combination satisfactory. More than these amounts caused dry, crumbly body. The difficulty was overcome by substituting dextrose for one-fourth of the sucrose.

Buttery, churned defects. Mortensen (93) stated in 1915 that pasteurization of the mix reduced viscosity, thereby lowering resistance to churning. As remedies he suggested holding the mix after pasteurization for 24 hours within a few degrees of freezing or homogenization. Baer (6) stated in 1918 that either centrifugal or steam emulsification made churning in the freezer more difficult. Ambrose (2) found that the use of 6 per cent milk-solids-not-fat increased the tendency of a mix to churn in the freezer.

Ice and sherbet defects. Dahlberg (28) in 1926 recommended replacement of 20 to 25 per cent of sucrose in sherbets with corn sugar to prevent crystallization of sucrose. He recommended 7 per cent corn sugar with 25 per cent sucrose as the best combination, and 0.2 per cent agar-agar with 0.4 per cent gum tragacanth or high grade India gum as a stabilizer. The author, with Penczek (27), later declared corn sugar or syrup essential for ices and sherbets. Smallfield (123) prevented crust formation on ices and sherbets by using 8 per cent honey with 22 per cent of sucrose.

Tuckey (146) stated in 1932 that two factors controlled hardness in ices and sherbets, sugar content and overrun, the former being the more important. Gums used alone caused crumbly body and excessive hardness. Gelatin caused excessive overrun but not crumbly body. Edman and Tracy (45) found that the new type of high conversion corn syrup, "Sweetose," containing increased dextrose and reduced dextrin, remained soluble in water when stored at $-15°$ F. It proved to be of decided advantage in preventing surface crustation on water ices. Tracy (138) improved body and texture of sherbets and ices by using "Sweetose" to replace 33 to 50 per cent of the sucrose normally used. Reid (111) stated in 1937 that sherbets served at 14° F. or higher were soggy. The Nebraska Experiment Station (101) reported in 1928 that sealing sherbet containers prevented sugar crustation at the surface. Parchment covers helped. Crustation was retarded materially by replacing a portion of the sucrose with invert or corn sugar. Gums proved unsatisfactory as stabilizers because of flavor.

Miscellaneous. Dahle and Josephson (36, 37) found that oat flour im-

proved body and texture. Martin and Caulfield (87) found that the use of 0.5 per cent of egg yolk in the mix did not affect body and texture adversely. Mueller and Button (96) found that 1 per cent dry egg yolk or dry whole egg improved texture while 1 per cent dry egg albumin injured texture. The improvement caused by dry egg yolk and dry whole egg was more noticeable in mixes of low solids content.

DEFECTS IN COLOR

Smith and Tracy (124) stated that consumers prefer a medium yellow color in ice cream. Ayres, Johnson, and Williams (3) found in 1918 that some grades of corn sugar imparted a yellow color. (Corn sugar in 1917–18 possessed a slight yellow color.) Ruehe (120) published that heating sucrose in the inversion process longer than 35 minutes darkened it, imparting to the syrup and the mix in which it was used an undesirable color. Tracy (136) and Corbett and Tracy (22) in 1936 found that corn sugar lowered color very slightly. Joslyn and Cole (65) found discolored frozen fruits affected adversely the color of ice cream.

Maybee (90) declared in 1939 that the finer cocoa is ground the deeper the color. Dahlberg (29) investigated in 1923 a greenish black discoloration in chocolate ice cream and found it to be due to chemical reaction between the tannins in cocoa or chocolate and exposed iron or rust spots on the containers. The discoloration appeared usually in the hardening room and occasionally in 1 to 10 days in the dealer's cabinet. The discolored ice cream tasted fishy. Alkaline, not acid, cocoa caused the difficulty. As remedies he suggested use of paper can liners, well-tinned containers, or, cocoa and chocolate free of tannins and alkali. Three out of eight samples examined contained tannins capable of entering into the chemical reaction.

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STUDIES ON THE PHOSPHATASE TEST AND ITS APPLICATION TO PROCESSED AND TREATED MILKS[1]

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The phosphatase test of Kay and Graham (1) has been modified and improved by several workers; has been used by many laboratories and health agencies supervising milk supplies; and in general has been found a very useful means of detecting imperfections and errors in the pasteurization of milk such as have been enumerated by Roger (2). A comprehensive review of the studies made of the phosphatase test and the results possible with its use, has been presented by Burgwald (3).

While a few investigations have been reported on the application of the phosphatase test to homogenized milk, preserved milk, chocolate milk, old milk and milk inoculated with various bacterial cultures, etc., (3, 4), there are a number of other practices, processes or modifications employed in the preparation and distribution of milk which might be expected to influence the application of the test. Some of these are incidental, such as contamination with metallic ions, exposure to light and development of oxidized flavor. Others are applied, such as irradiation, addition of vitamin D concentrates, homogenization and enzyme treatment. The investigation here reported was for the purpose of determining the effect of such things on the accuracy of the test. In the course of the studies variations in results were noted due to the time interval allowed for development of color following the addition of Gibbs' indicater (BQC). There seems to be some difference of opinion concerning the rapidity with which the color reaction takes place and its completeness. For this reason the matter was given some attention in an effort to evaluate its importance.

EXPERIMENTAL METHODS

Scharer's laboratory modification (5, 6) of Kay and Graham's phosphatase test was employed in this investigation because it requires less time, the reagents are not so difficult to prepare and the indicator (Gibbs' phenol

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reagent) is more specific for phenols than is Folin and Ciocalteau's reagent (3, 4, 7). Scharer's method calls for an incubation period of one hour at 95–115° F. (35–46° C.) but it has been suggested that greater sensitivity can be obtained with a two-hour period and a temperature over 100° F. (37.8° C.). In this study the samples were incubated at 105° ± 0.2° F. (40.5° C. ± 0.1° C.) for two hours. After the addition of the indicator to the test solutions, the intensity of color developed was determined at the end of exactly fifteen minutes and again after exactly thirty minutes.

In preliminary studies, a visual colorimeter was used to determine the intensity of color developed. In the investigation proper a Klett-Summerson photoelectric colorimeter (photelometer) was used with more satisfactory results than could be had with the visual colorimeter.

For some of this work, it was deemed desirable to simulate commercial conditions of pasteurization as closely as possible. In such cases, one quart samples were pasteurized in a one-liter, three-necked, round-bottom flask, fitted with an electric stirrer and an accurate thermometer. Preheating required nine to ten minutes in a separate bath held at 155–160° F. (68.8–71.0° C.) after which the flask was transferred to a constant temperature bath. It was possible to hold a sample at any given temperature, plus or minus 0.1° F. (0.05° C.). When it was necessary to remove samples at intervals, they were taken by means of a 9-ml. cream pipette, placed in test tubes and cooled in running tap water.

In other cases where it was impractical to pasteurize an entire set of samples individually in the flask and only a comparison between samples was desired, they were pasteurized in test tubes in the constant temperature bath. Preheating in such cases was also accomplished in the bath in three to five minutes.

EXPERIMENTAL RESULTS, PART I

Calibration of the Photelometer. A suitable light filter for use with the Klett-Summerson photelometer was determined by means of a Coleman spectrophotometer. The typical blue color was developed in 5 ml. of a 0.5 p.p.m. solution of phenol by the addition of 0.25 ml. of the borate buffer[2] and two drops of BQC (2,6 dibromoquinonechloroimide), allowing 30 minutes for color development. The percentage transmission of light was then measured using wave lengths at 30 millimicron intervals from 350 to 880 millimicrons. The least transmission of light (region of greatest sensitivity) was found in the band having a median wave length of 600 milimicrons. A number 66 filter (660 millimicrons) was immediately available and since this difference in wave length did not materially affect the sensitivity, it was used in this study.

A calibration curve for the purpose of converting subsequent phospha-

[2] 0.5 ml. is now recommended.

tase test readings to phenol equivalents (parts per million) was obtained for the Klett-Summerson photelometer as follows:

A series of standard phenol solutions containing from 0.1 to 10 p.p.m. of phenol were prepared following the method used by Kay and Graham (1). Color standards (5) were prepared by placing 5 ml. of the above solutions in colorimeter tubes, adding 0.25 ml. of buffer and at intervals of thirty seconds, adding 2 drops of BQC indicator to each tube in turn. Photelometer readings were made after exactly 15- and 30-minute periods of color development.

The average photelometer readings for four determinations are given in table 1 and are shown graphically in figure 1.

TABLE 1

Photelometer readings on phenol color standards

Phenol	Color development period	
	15 min.	30 min.
	Photelometer readings	
p.p.m.		
0.1	17.0	23.1
0.2	24.4	31.7
0.3	33.3	41.4
0.4	42.6	51.2
0.5	49.5	59.2
0.6	58.8	68.1
0.7	66.7	76.9
0.8	75.5	86.4
0.9	82.8	95.2
1.0	91.9	105.0
2.0	134.0	178.0
3.0	183.0	244.0
4.0	227.0	298.0
5.0	255.0	319.0
7.0	296.0	345.0
10.0	329.0	358.0

The scale of the Klett-Summerson photelometer is graduated logarithmically so that the data should lie along a straight line if the development of color is in accordance with Beer's Law. That this is true for the solutions from 0.1 to 1.0 p.p.m. of phenol may be seen in the enlarged section of figure 1. At higher concentrations of phenol, color development is less than would be expected from Beer's Law, the 15-minute color development curve deviating at a lower phenol value than the 30-minute curve.

These data indicate that color development in phenol solutions is not complete in 15 minutes, particularly at the higher phenol levels but is significantly incomplete even at the lower levels.

Influence of Time Allowed for Development of Color. Gibbs (7) has shown that the intensity of color in a phenol solution treated with BQC

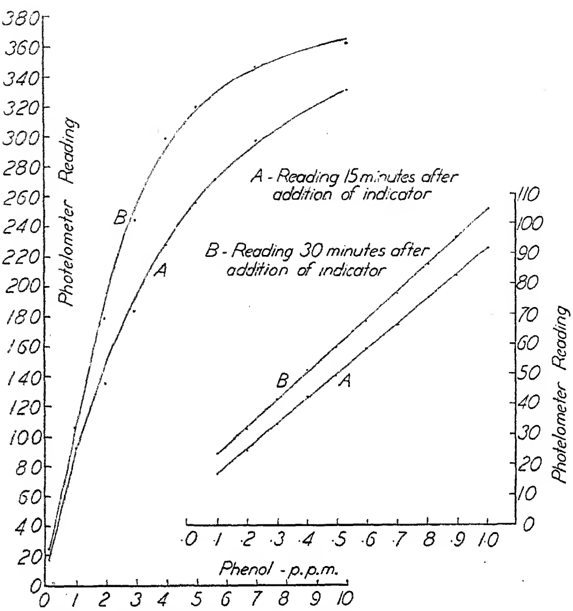

FIG. 1. Calibration curve for the phosphatase test. Phenol standards, 0.1 to 10.0 p.p.m. of phenol.

indicator is a function of the time allowed for color development. Scharer's method (5, 6) stipulates a color development period of 15 minutes and it is inferred that the maximum color is developed in this length of time. A study was made of the color development in a 0.40 p.p.m. solution of phenol using a Coleman spectrophotometer. The curve obtained, plotting per cent transmittance of light against time is presented in figure 2.

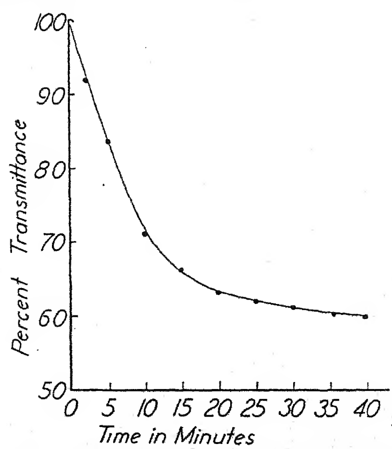

FIG. 2. Intensity of color developed in a 0.4 p.p.m. solution of phenol as affected by time allowed for development.

It may be seen that the development of color in a very dilute solution of phenol is almost complete at the end of 30 minutes but not at the end of 15 minutes. The time allowed for color development in solutions of phenol is obviously an important factor in the calibration of a photelometer for use with the phosphatase test. This was confirmed for the development of color in phosphatase tests with milk as well as with solutions of phenol.

Mixed herd milk was pasteurized in test tubes at 141°, 142°, 143°, 144° and 145° F., samples being removed after holding intervals of 20, 25 and 30 minutes, respectively. Photelometer readings were made on these samples, after applying the phosphatase test, and after allowing 15, 30, 45 and 60 minutes, respectively, for the development of color following the addition of the BQC indicator. The results of this study are shown in table 2 and the data for two of the pasteurization treatments (143° and 145° F.) are presented graphically in figure 3.

TABLE 2

Influence of color development period upon the intensity of color produced in the phosphatase test

Pasteurization		Color development period—minutes			
Temperature	Time	15	30	45	60
		Photelometer readings			
°F.	*min.*				
141	20	248	276	276	276
	25	147	220	228	228
	30	95	151	166	165
142	20	145	216	222	222
	25	80	129	140	143
	30	49	80	92	95
143	20	64	104	117	121
	25	39	62	72	74
	30	33	50	62	65
144	20	48	77	90	93
	25	29	46	55	57
	30	23	37	45	47
145	20	33	51	59	61
	25	25	40	47	51
	30	21	36	43	48

Where the amount of phenol, resulting from the action of the phosphatase enzyme, is small, the intensity of color increases for at least sixty minutes. More concentrated solutions require a shorter time to reach a maximum color. A comparison of the data shown in figure 2 with those presented in figure 3, furthermore, indicates that a constant color is approached in a shorter time with pure phenol solutions than where the phenol results from the action of the phosphatase enzyme of the milk. These findings further emphasize the fact that the phosphatase test must be considered

a conventional test requiring definite standardization of the procedure with respect to the time allowed for color development in the standards used for comparison as well as in the unknowns. This would be true regardless of the type of color comparator employed.

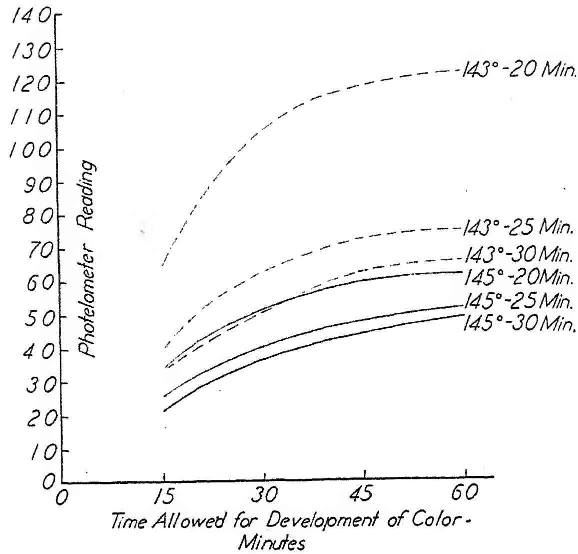

FIG. 3. Influence of time allowed for development of color upon the intensity of color produced using variously heated milk samples.

Effect of Time and Temperature of Pasteurization on Phenol Values of Milk. The practical value of the phosphatase test is dependent upon its ability to detect minor discrepancies in the pasteurization process. Other investigators (3, 5, 8, 9, 10), using Scharer's method, have generally agreed that the test is capable of detecting the following errors in pasteurization, assuming the legal standard to be 143° F. for 30 minutes: a 5 minutes shorter holding time; a lower temperature of one degree; or the addition of one-tenth of one per cent of raw milk. The same investigators, however, have not been able to agree upon the correct phenol value to set as a dividing line between properly and improperly pasteurized milk. Scharer (5) at first stated that milk pasteurized at 143° F. for 30 minutes gives a phenol value not greater than 0.5 p.p.m., but later (6) reduced this figure to 0.4 p.p.m. Other investigators have indicated Scharer's value to be too low. Tracy and Hahn (10) first set the dividing line at 0.6 p.p.m. then changed it to 0.8 p.p.m. (9). The work of Burgwald and Giberson (8) indicated 0.9 p.p.m. as the critical value.

A study was made of the progressive destruction of phosphatase by pasteurization of one-quart samples of mixed herd milk at temperatures

from 142 to 145° F. Samples were taken before pasteurization and at the end of 5, 10, 15, 20, 25 and 30 minutes of holding at the specified temperatures, ± 0.1° F. They were then cooled in ice water. The time of preheating varied from 9 to 10 minutes. The average phenol values of triplicate determinations are presented in table 3 and shown graphically in figure 4. The raw milk gave an average phenol value of 7.7 p.p.m. when 15 minutes were allowed for color development and the photelometer readings were referred to the 15-minute phenol standard. The average phenol value was 4.4 p.p.m. using a 30-minute color development period and referring the readings to the 30-minute phenol standard.

TABLE 3

Effect of pasteurization temperature and holding time upon the phenol values of milk (expressed in parts per million of phenol)

Holding time	Pasteurization temperature			
	142° F.	143° F.	144° F.	145° F.
	Color developed for 15 minutes and referred to the 15-minute phenol standard			
min.				
5	6.0	5.5	4.7	4.8
10	5.0	4.3	2.3	1.0
15	3.5	1.8	0.67	0.32
20	1.8	0.65	0.25	0.15
25	0.85	0.31	0.13	0.11
30	0.51	0.18	0.09	0.10
	Color developed for 30 minutes and referred to the 30-minute phenol standard			
5	3.5	3.3	3.0	3.0
10	2.7	2.8	2.0	1.2
15	2.4	1.8	0.82	0.40
20	1.3	0.85	0.34	0.20
25	1.0	0.43	0.18	0.16
30	0.61	0.28	0.12	0.15
	Average phenol value of the raw milk was 7.7 p.p.m. with a 15-minute period for color development and 4.4 p.p.m. with a 30-minute period.			

These data demonstrate that the phosphatase test is more accurate in detecting discrepancies in pasteurization with the lower than with the higher temperatures. It is possible to detect underheating of 5 minutes at 142 or 143° F., of 10 minutes at 144° F. and of 15 minutes at 145° F. It is also possible to detect too low a temperature of one degree when the standard is 143 or 144° F. and of two degrees when the standard is 145° F. The results further indicate that the following heat treatments of milk are equally destructive of phosphatase: 143° F. for 30 minutes; 144° F. for 23 minutes; and 145° F. for 19 minutes.

A comparison of the upper and lower parts of table 3, or the two sets of curves in figure 4, reveals a very definite difference in phenol values obtained

using 15 minutes for color development as compared with 30 minutes. Where the amounts of phenol present are relatively large (over 1.5 p.p.m.) the 15-minute period of color development produces higher phenol values than the 30-minute period, whereas the opposite is true when the amounts of phenol are relatively small. From a practical standpoint, either procedure may be used with only a slight shift in the phenol value representative of proper pasteurization but tests made without careful control of the period allowed for color development cannot be depended upon.

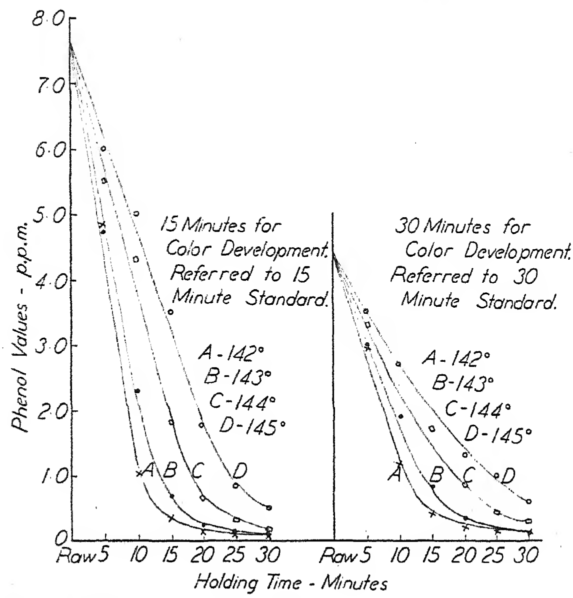

FIG. 4. Effect of pasteurization temperature and holding time upon the phenol value of milk.

The practical use of the phosphatase test necessitates the designation of an arbitrary value as the dividing line between properly and improperly pasteurized milk. This study indicates that milk pasteurized at 143° F. for 30 minutes has a phenol value of less than 0.25 p.p.m. when a 15-minute color development period is used and the photelometer readings are referred to the 15-minute phenol standard. However, when a 30-minute period is used and the readings are referred to the 30-minute phenol standard, the same milk has a phenol value of somewhat less than 0.40 p.p.m. This latter procedure is, of course, a digression from Scharer's published method and was used only to illustrate the importance of the length of time allowed for color development.

It is difficult to adequately explain the difference of opinion among the various investigators concerning the correct phenol value representative of

proper pasteurization. No investigator has mentioned the importance of the length of time allowed for color development in the color standards and in the phosphatase test and it is believed that variations in this factor may be largely responsible for the dissimilarity of the results obtained. Burgwald and Giberson (8) suggest that variations in sensitivity of the BQC indicator may be a factor. Another contributing cause may be the different methods of standardizing the phenol solutions and of preparing the phenol color standards.

Effect of the Addition of Raw Milk to Pasteurized Milk on the Phenol Value. Studies were made as to the effect of additions of small quantities of raw milk to milk properly pasteurized at 142° F. to 145° F. on the phenol values obtained with the phosphatase test. The results indicated that quantities of raw milk as small as 0.1 per cent can be definitely detected where the temperature of pasteurization is known, increases in the phenol value for a given amount of raw milk being relatively uniform regardless of the pasteurization temperature. Detection is more certain using a 15-minute period for color development since the phenol values are lower.

EXPERIMENTAL RESULTS, PART II

In the following studies the photelometer readings were made on all samples after a 15-minute period for color development and were referred to the 15-minute phenol standard for conversion into p.p.m. of phenol.

Influence of Fortification of Milk with Vitamin D on the Phosphatase Test. No information is available in the literature to indicate whether the phosphatase test is applicable to vitamin D reinforced milks. While it would probably not be expected that the addition of vitamin D concentrates would have any effect upon the accuracy of the test, it is conceivable that the activity of the phosphatase enzyme might be affected by the treatment of milk with ultraviolet radiations. Studies were made with irradiated milk and with milk reinforced with Vitex, Clo-Dee and A.R.P.I. vitamin D concentrates.

Irradiation. Two series of commercially pasteurized milk samples were obtained from different sources. Each consisted of three sets of samples from different lots of milk, one portion being irradiated subsequent to pasteurization; the other portion, unirradiated. The treatment of the samples and the resulting phenol values of phosphatase tests are presented in table 4.

Vitamin D Concentrates. Other series of samples were prepared in the laboratory from mixed herd milk using vitamin D concentrates and were pasteurized at 143° F. for 30 minutes. Samples were made up to contain 400 and 800 International Units per quart from four concentrates, Vitex, Clo-Dee, A.R.P.I. No. 6 and A.R.P.I. No. 15. The phenol values obtained in phosphatase tests are shown in table 5.

The data indicate rather conclusively that milk may be reinforced with

TABLE 4

Influence of irradiation on the phenol values of milk (expressed in p.p.m. of phenol)

Samples	145° F.—30 min.*		160° F.—16 sec.*		143° F.—30 min.†	
	Not irradiated	Irradiated 135 I.U. Vit. D	Not irradiated	Irradiated 135 I.U. Vit. D	Not irradiated	Irradiated 400 I.U. Vit. D
1st set	0.11	0.13	0.09	0.07	0.11	0.105
2nd set	0.10	0.10	0.10	0.10	0.10	0.105
3rd set	0.14	0.15	0.13	0.13	0.10	0.09

* Samples made available through the courtesy of F. M. Greenleaf of the Lehigh Valley Cooperative Farmers Association, Allentown, Pa.

† Samples obtained through the cooperation of Dr. K. G. Weckel, Department of Dairy Industry, University of Wisconsin.

TABLE 5

Influence of adding vitamin D concentrates before pasteurization on the phenol values of milk

Samples	Vitex	Clo-Dee	A.R.P.I. No. 6	A.R.P.I. No. 15
Control—No concentrate	0.105	0.125	0.125	0.13
400 I.U. per quart	0.115	0.14	0.13	0.13
800 I.U. per quart	0.09	0.11	0.13	0.15

vitamin D by irradiation or by the addition of vitamin D concentrates without affecting the accuracy of the phosphatase test.

Influence of Pancreatic Enzymes Added to Milk before Pasteurization on the Phosphatase Test. The addition of a pancreatic extract has been proposed (11) as a means of lowering the curd tension of milk. The extract is available under the name Enzylac and is used by introducing a very small amount into milk, allowing a short period of time for enzymatic action and then immediately pasteurizing. The pasteurization destroys the enzymes and no further action takes place. The use of this extract in a similar manner at the rate of one gram of extract to each ten to forty gallons of milk, has also been suggested (12) as an effective means of preventing oxidized flavor. This practice is illegal in most states but is being used in some places with or without the sanction of the health agencies.

To determine whether the presence or the action of the enzymes might have an effect upon the phosphatase test, a series of samples was prepared from mixed herd milk containing the recommended concentration of enzyme and pasteurized at 143° ± 0.1° F. Table 6 shows the phenol values obtained in phosphatase tests from these samples.

It is apparent that the addition of pancreatic extract (Enzylac) to milk before pasteurization in amounts necessary to produce soft curd milk or to prevent oxidized flavor has no effect upon the phosphatase test.

Influence of Contamination of Milk with Small Quantities of Metallic Ions on the Phosphatase Test. During the handling and pasteurization

TABLE 6

Influence of additions of pancreatic enzyme to milk before pasteurization on the phenol values

Concentration of enzyme	Phenol
	p.p.m.
None	0.13
1 gram/40 gal.	0.14
1 gram/10 gal.	0.14

process, milk necessarily picks up traces of metallic ions and holds them in solution. Since enzymes are known to be activated by certain ions and inhibited by others, a study was made to ascertain what effect, if any, the following ions have on the phosphatase test: copper, nickel, iron, aluminum and tin. The ions were added to the mixed herd milk in concentrations up to ten parts per million and the samples were pasteurized in test tubes at 143° F. for 30 minutes. The average phenol values of duplicate phosphatase determinations, are presented in table 7.

TABLE 7

Phenol values of milk contaminated with metallic ions before pasteurization (expressed in p.p.m. of phenol)

Concentration of ion	Ions added				
	Cu	Ni	Fe	Al	Sn
p.p.m.					
0	0.12	0.15	0.12	0.15	0.13
0.5	0.11	0.13	0.12	0.13	0.16
1.0	0.12	0.15	0.11	0.17	0.17
3.0	0.13	0.12	0.10	0.14	0.17
5.0	0.12	0.12	0.10	0.16	0.17
7.0	0.11	0.10	0.09	0.15	0.13
10.0	0.11	0.11	0.11	0.13	0.13

Similar results were obtained with samples which were contaminated with these ions after pasteurization. Apparently metallic contamination such as might occur in the normal handling of milk exercises a negligible effect on the phosphatase test.

Influence of the Development of Oxidized Flavor in Milk on the Phosphatase Test. A one quart sample of mixed herd milk was pasteurized at 143° ± 0.1° F. for 30 minutes and divided into three parts. The first portion was used as a control; copper was added to the second at the rate of two p.p.m.; the third was exposed to bright sunlight for one hour. The sample containing copper was oxidized at the end of 24 hours and became progressively worse. The sunlight sample developed a typical burnt or activated flavor. The samples were tested immediately and at the end of 24, 48, and 72 hours storage at 40° F. Table 8 shows the average phenol values of triplicate phosphatase determinations.

TABLE 8

Influence of the development of oxidized flavor in milk on the phenol value
(Expressed in p.p.m. of phenol)

Samples	Storage period			
	Immediately	24 hrs.	48 hrs.	72 hrs.
Control	0.10	0.14	0.15	0.16
Cu. 2 p.p.m.	0.10	0.13	0.16	0.16
Sunlight	0.10	0.12	0.14	0.15

These data indicate no significant difference between the control and the treated samples. While there is a more or less regular increase in phenol value for all of the samples, it is too slight to be significant and may be due to experimental variations.

Other Studies. Homogenization of milk was found to have no influence on the phosphatase test as has been noted by other investigators (3, 9, 13). Studies on milk held for periods up to six days under refrigeration indicate that, as others have shown (5, 8, 14, 15, 16), the age of milk does not affect the accuracy with which the phosphatase test may be applied. Data for these studies are omitted.

CONCLUSIONS

1. A photoelectric colorimeter (photelometer) offers a convenient and accurate means of determining the intensity of color produced by the phosphatase test. It has the advantage of permitting an exact period of time for color development, and phenol standards, which are unstable, need be prepared only once.

2. The proper filter to use with Scharer's modification of the phosphatase test, if color intensities are to be determined with a photelometer, is one which will exclude practically all of the light except that in a rather narrow band of the spectrum having a median wave length of approximately 600 millimicrons.

3. The development of color in dilute solutions of phenol, using 2,6,-dibromoquinonechloroimide (BQC) as an indicator, is practically complete at the end of thirty minutes but not at the end of fifteen minutes. Therefore, in the calibration of a photelometer, the period of time allowed for color development in the standard phenol solutions should be carefully controlled.

4. Phosphatase tests made on milk samples containing relatively small quantities of active phosphatase enzyme, increase in color intensity for at least sixty minutes. More concentrated solutions require a shorter time to reach a maximum color. It is essential to allow a uniform carefully controlled period of time for color development in the performance of the phosphatase test and to make photelometer readings at exact intervals.

5. The phosphatase test is capable of detecting underheating of 5 minutes at 142 and 143° F., of 10 minutes at 144° F., and of 15 minutes at 145° F.

It is capable of detecting too low a temperature of one degree when the pasteurization standard is 143 or 144° F. and of two degrees when the standard is 145° F. As little as one-tenth of one per cent of raw milk may be detected when added to milk which is assumed to have been pasteurized at any specific temperature between 142 and 145° F. for 30 minutes.

6. The accuracy of the phosphatase test as applied to pasteurized milk is not affected to a significant extent by any of the following treatments or processes:

a. Reinforcement of milk with vitamin D either by irradiation or by the addition of vitamin D concentrates;
b. Addition of pancreatic extract (Enzylac) for producing a soft curd type of milk or for preventing oxidized flavor;
c. Contamination with such metallic ions as copper, nickel, iron, aluminum, or tin in concentrations up to ten parts per million;
d. Development of oxidized flavor;
e. Homogenization;
f. Storage under refrigeration for periods as long as six days.

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MASTITIS. I. THE RELATIONSHIP OF THE DEVELOPMENT OF MASTITIS TO CHANGES IN THE CHLORINE, LACTOSE AND CASEIN NUMBER OF MILK*

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The secretion of milk of abnormal composition by cows with latent or chronic mastitis has been generally recognized for a long time. In 1937 Hastings and Beach (4) summarized the prevailing opinion that approximately ninety per cent of the cases of chronic mastitis are due to *Streptococcus agalactiae* and the remainder to other bacteria such as various species of *Streptococcus, Staphylococcus, Micrococcus* and varieties of *Escherichia coli.*

The most apparent chemical changes in milk due to mastitis or associated with mastitis are decreases in the fat, solids-not-fat, casein, lactose, ash and titratable acidity, and increases in water, non-casein nitrogen, chlorine and pH value. Certain of these chemical constituents have been determined in milk from diseased udders and compared with milk from non-infected udders as an indirect biochemical method for detecting mastitis. The chemical constituents most commonly determined for this purpose are the chlorine, the lactose, and the pH value of the milk. Normal milk is said to contain less than 0.14 per cent chlorine, at least 4 per cent lactose, and to have a pH value below 6.8 (4, 6).

Factors other than mastitis such as an increase in the number of lactations, the stage of lactation and a decrease in the solids-not-fat content of the milk are known to cause an increase in the percentage of chlorine in the milk (3, 5, 8, 15). There is also evidence that milk from udders free of mastitis may at times contain more than 0.14 per cent chlorine (3, 4, 5, 10). Hastings and Beach (4) have also demonstrated wide differences in the chlorine, acidity, catalase and pH values of milk depending upon whether the sample was fore-milk or whole milk. Johns and Hastings (7) observed a marked fluctuation, often of a rhythmic character, in the chlorine, catalase and pH value for fore-milk from milking to milking. Infected quarters as shown by the presence of *Streptococcus* were found frequently to yield normal milk while non-infected quarters yielded milk giving definite abnormal reactions. These investigators stressed the need of caution in the use of indirect tests as the basis for diagnosis of mastitis and emphasized

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the value of examining a series of samples at consecutive milkings in order to obtain a true picture of the condition of the quarter. Davis (3) found that the percentage of chlorine was very uniform in the milk of cows in good health, whether the period of sampling was monthly, weekly, or daily, but milk high in chlorine showed considerable variation when sampled daily or weekly.

Koestler (9) proposed the chlorine-lactose number

$$\left(\frac{\text{per cent chlorine}}{\text{per cent lactose}} \times 100\right)$$

for detecting mastitis. In cases of mastitis the chlorine increases whereas the lactose decreases, therefore the chlorine-lactose number changes proportionately more than either chlorine or lactose alone.

Rowland (12) made an extensive study of the protein distribution in normal and abnormal milks, and found that cows with sub-clinical mastitis can be detected by determining the "casein number" which is the percentage casein nitrogen of the total nitrogen in the milk.

Rogers and associates (2) reviewed a limited number of studies made on the composition of milk from individual quarters of the same udder. The results indicate wide differences in the chemical composition of milk from healthy and diseased quarters of the same udder.

Since various uncontrollable factors other than the health of the udder are known to affect the chemical composition of milk, it seemed desirable to make a study of the composition of milk from individual quarters of the same udder for entire lactation periods. By studying several constituents in the milk, such as the chlorine, lactose and nitrogen distribution or "casein number," it was thought that possibly one constituent might show a significant change at an earlier stage in the development of mastitis than the others, thus making it possible to increase the accuracy of an indirect biochemical test for the diagnosis of mastitis.

EXPERIMENTAL

This investigation was started in the fall of 1938 in connection with a study of the possible relationship of certain constituents in the ration and the method of feeding as predisposing factors to the development of mastitis. When the investigation was started, twenty-two pure bred Holstein cows were available for observation. Of the twenty-two cows, seven were in the latter part of the first lactation, six were in the second lactation, two in the third, five in the fourth, one was in the fifth and one in the sixth lactation. During the course of the study eighteen pure-bred Holstein heifers in their first lactation were added to the herd making a total of forty animals under observation.

For several months preceding the present study and throughout the investigation, routine physical examinations of the udder and the following

diagnostic tests were made at biweekly intervals on samples of fore-milk; strip cup, brom-thymol blue, chlorine (colorimetric), Hotis test, leucocyte count, microscopic examination, and blood agar plate.

The diagnosis of mastitis was based upon the presence of hemolytic streptococci accompanied by changes indicated by one or more of the other tests, which supported the bacteriological findings. The presence of occasional hemolytic streptococci, or other organisms particularly hemolytic staphylococci, as well as non-hemolytic streptococci and staphylococci in large numbers, accompanied by changes indicated by the other tests that show evidence of mastitis, was considered as suspicious. If tests other than the blood agar plate showed an increase in the severity of reaction, such animals were considered highly suspicious and usually on subsequent tests were found positive.

When this investigation was started seven of the twenty-two cows were diagnosed as having latent or chronic mastitis in one or more quarters. During the period covered by this study several additional animals were diagnosed as positive or suspicious with regard to mastitis.

Samples of about six to eight ounces of fore-milk were collected at monthly intervals from each quarter at the morning milking. The samples were taken to the laboratory and analyzed for chlorine, lactose, total nitrogen and non-casein nitrogen on the same day that the samples were collected. Total nitrogen and non-casein nitrogen were determined and the casein number was calculated as described by Rowland (11, 12), except that larger samples were used and the determinations were made using regular macro Kjeldahl apparatus. Chlorine was determined according to the method of Sanders (14), except that 10 ml. of the sample with the required amount of the silver nitrate solution were diluted to 100 ml. in a volumetric flask and filtered. The excess silver nitrate was titrated in 25-ml. aliquots of the filtrate. Lactose was determined by the A.O.A.C. optical method (1), and the chlorine-lactose number was calculated as was outlined by Koestler (9).

RESULTS

In connection with this study about 250 udder examinations were made by chemical analyses of milk from individual quarters. The individual quarters were divided into three groups according to the physical condition of the quarter and the reaction of the various diagnostic tests made during the month in which the samples of milk were taken for chemical analysis. Group I included quarter samples from udders in which all quarters were negative. Group II included samples from negative quarters from udders in which one or more quarters were positive. Group III included samples from positive quarters.

A summary of the data obtained for the three groups is presented in table 1.

TABLE 1

The chlorine, lactose, chlorine-lactose number, and casein number for the individual quarters in the various groups

Diagnosis of quarters	No. of animals	No. of quarter samples	Minimum	Maximum	Mean ± S.E.	Coeff. of Var.
			Per cent chlorine			
Group I. All quarters negative in udder	26	376	0.090	0.224	0.124 ± 0.001	8.06
Group II. Neg. quarters from pos. udders	15	142	0.089	0.239	0.119 ± 0.002	18.49
Group III. Pos. quarters from pos. udders	18	201	0.087	0.273	0.150 ± 0.003	26.00
			Per cent lactose			
Group I. All quarters negative in udder	18	296	3.30	5.40	4.788 ± 0.007	2.36
Group II. Neg. quarters from pos. udders	14	121	4.00	5.75	4.872 ± 0.033	7.51
Group III. Pos. quarters from pos. udders	17	146	2.30	5.40	4.347 ± 0.065	18.15
			Chlorine-lactose number			
Group I. All quarters negative in udder	18	296	1.62	6.79	2.614 ± 0.027	17.71
Group II. Neg. quarters from pos. udders	14	121	1.67	4.70	2.457 ± 0.057	25.36
Group III. Pos. quarters from pos. udders	17	146	1.83	10.95	3.665 ± 0.149	49.25
			Casein number			
Group I. All quarters negative in udder	26	316	69.4	84.3	77.57 ± 0.11	2.56
Group II. Neg. quarters from pos. udders	15	137	68.6	83.1	77.76 ± 0.22	3.24
Group III. Pos. quarters from pos. udders	18	198	56.8	84.1	74.15 ± 0.32	6.08

From a study of the data presented in table 1 it may be seen that the minimum and maximum values obtained for the various groups are on the whole very similar. This illustrates the difficulty in the diagnosis of mastitis by changes in the chlorine, lactose, chlorine-lactose number or casein number of the milk when some arbitrary value is used as a criterion above or below which is taken as indicating mastitis. The means for the negative quarters are very similar even though one or more quarters in the same udder may have been diagnosed as positive but are significantly different from the means for the positive quarters. The means for the positive quarters show the effect of abnormal milk in this group, as the percentage chlorine was higher and the percentage lactose was lower than for the negative quarters. The means for the chlorine-lactose number was higher and the casein number lower for the positive quarters than the means for the

negative quarters. There was a successive increase in the coefficient of variation from the first to the third group. The chlorine-lactose number was subject to the greatest variation of any of the constituents studied. The percentage chlorine shows the next greatest variation, followed by the percentage lactose with the casein number showing the least amount of variation.

From physical, bacteriological and chemical evidence it is known that mastitis does not develop in the four quarters of an udder simultaneously, but generally is first observed in a single quarter and then may spread to other quarters of the udder. The milk from unaffected quarters usually appears normal in composition. If it is assumed that the milk from unaffected quarters is normal in composition, then by a comparison of the chemical composition of the milk from the individual quarters of the same udder it should be possible to eliminate to a considerable extent the effect of variation in the composition of milk due to uncontrollable factors such as season, number and stage of lactation as well as the individuality of the animal, thus making the diagnosis of mastitis by changes in the chemical composition of the milk much more accurate. In order to determine the extent of variation which might be expected among quarters of udders that were free of mastitis a study was made of the differences between quarters numbers 1[4] and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4, and 3 and 4 for all udders regardless of number and stage of lactation. For the study the following constituents were considered: chlorine, lactose, chlorine-lactose number, and casein number. In table 2 is presented, in summary form, the number of normal udder examinations, the number of animals involved, and the number of differences between quarters upon which the means and standard deviations are based. The differences between quarters were considered as individual variates in a normal distribution. The means and standard deviations were determined as usual. For a single constituent in the milk of one quarter to be considered significantly different from that of any other

TABLE 2

Number of normal animals examined, number of udder examinations, number of quarter differences—mean of differences, standard deviation and greatest difference permissible without being a significant difference between quarters

Constituent	No. of animals	No. of normal udder examinations	No. of quarter differences	Mean of differences	S. Dev.	Mean plus 2 S. Dev. (Odds 1: 19)
Chlorine	26	80	480	0.0065	0.0066	0.020
Lactose	18	74	444	0.1154	0.1234	0.362
Chlorine-lactose No.	18	74	444	0.1842	0.2081	0.600
Casein No.	26	79	474	0.9987	0.9512	2.901

[4] R.F. quarter No. 1, R.R. quarter No. 2, L.R. quarter No. 3, L.F. quarter No. 4.

TABLE 3

Variation from milking to milking for chlorine, lactose, and chlorine-lactose number for one normal animal and three animals with chronic mastitis

No. of animal	Quarter	Time of sampling					
		First day		Second day		Third day	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
		Per cent chlorine					
475	1	0.108	0.116	0.102	0.106	0.105	0.110
	2	0.120	0.124	0.103	0.120	0.109	0.118
	3	0.108	0.110	0.101	0.107	0.101	0.106
	4	0.106	0.110	0.106	0.106	0.104	0.106
		Per cent lactose					
	1	5.25	5.15	5.10	5.25	5.20	5.15
	2	5.10	4.95	5.10	5.05	5.15	5.05
	3	5.25	5.05	5.20	5.20	5.25	5.15
	4	5.25	5.20	5.20	5.15	5.30	5.15
		Chlorine-lactose number					
	1	2.06	2.25	2.20	2.02	2.02	2.14
	2	2.35	2.51	2.02	2.38	2.12	2.34
	3	2.06	2.18	1.94	2.06	1.92	2.06
	4	2.02	2.12	2.04	2.06	1.96	2.06
		Per cent chlorine					
469	1	0.178*	0.166*	0.175*	0.174*	0.183*	0.168*
	2	0.131	0.139	0.136	0.140	0.138*	0.122
	3	0.162*	0.150*	0.146*	0.150*	0.146*	0.136
	4	0.124	0.122	0.122	0.122	0.118	0.122
		Per cent lactose					
	1	3.90*	3.60*	3.65*	3.95*	3.75*	3.90*
	2	4.80	4.55	4.75	4.50	4.60*	4.80
	3	4.05*	4.30*	4.35*	4.35*	4.35*	4.45
	4	4.95	4.75	4.80	4.80	5.00	4.65
		Chlorine-lactose number					
	1	4.56*	4.61*	4.79*	4.41*	4.88*	4.31*
	2	2.73	3.05	2.86	3.11	3.00*	2.54
	3	4.00*	3.49*	3.36*	3.45*	3.36*	3.06
	4	2.51	2.57	2.54	2.54	2.36	2.62
		Per cent chlorine					
486	1	0.132*	0.135*	0.125	0.162*	0.138*	0.154*
	2	0.115	0.117	0.114	0.120	0.117	0.161*
	3	0.110	0.115	0.111	0.116	0.113	0.136
	4	0.116	0.114	0.113	0.125	0.117	0.130
		Per cent lactose					
	1	4.80	4.75	4.70	4.25*	4.65*	4.65
	2	5.15	5.10	4.90	5.00	5.10	4.75
	3	5.15	4.95	5.00	5.10	5.20	4.90
	4	5.10	4.80	4.85	4.85	4.95	4.85
		Chlorine-lactose number					
	1	2.75*	2.84	2.66	3.81*	2.97*	3.31*
	2	2.23	2.29	2.33	2.40	2.29	3.39*
	3	2.14	2.32	2.22	2.27	2.17	2.78
	4	2.27	2.38	2.33	2.58	2.36	2.68

TABLE 3.—(*Continued*)

No. of animal	Quarter	Time of sampling					
		First day		Second day		Third day	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
		Per cent chlorine					
	1	0.152*	0.162*	0.158*	0.152	0.158*	0.136
	2	0.160*	0.160*	0.152*	0.173*	0.178*	0.134
	3	0.136	0.135	0.131	0.141	0.133	0.119
	4	0.124	0.130	0.127	0.133	0.124	0.122
		Per cent lactose					
490	1	4.35	4.30	4.25	4.45	4.40*	4.40
	2	4.30	4.25	4.15	4.10*	4.05*	4.20*
	3	4.55	4.55	4.50	4.40	4.70	4.60
	4	4.70	4.60	4.70	4.65	4.90	4.70
		Chlorine-lactose number					
	1	3.49*	3.77*	3.72*	3.42	3.59*	3.09
	2	3.72*	3.76*	3.66*	4.22*	4.39*	3.19*
	3	2.99	2.97	2.91	3.20	2.83	2.59
	4	2.64	2.83	2.70	2.86	2.53	2.60

* Abnormal chemical composition.

quarter with which it might be compared, the difference must be equal to or greater than the mean of the quarter differences plus two times the standard deviation.

From the data presented in table 2 it may be seen that for one quarter to be considered significantly different from any other quarter in the same udder with which it might be compared, there must be a difference of as much as 0.02 per cent in chlorine, and 0.36 per cent in lactose. There must also be a difference of 0.60 in the chlorine-lactose number and a difference of 2.90 in the casein number. These differences include variations in sampling and in analytical methods, as well as normal variations in the composition of milk from udders free from disease. For these differences the odds are 1 to 19, which allows a difference as great as these in 5 per cent of the cases and still be within the normal range of variation. The odds increase very rapidly when the differences between quarters exceed to an appreciable extent the limits considered as significant. In order to detect significant changes in the chlorine, lactose, chlorine-lactose number, or casein number of the milk due to or associated with mastitis by a comparison of quarter differences, the quarter with the lowest percentage chlorine and chlorine-lactose number, or highest percentage lactose and casein number is considered normal.

A study of the effect of mastitis on the composition of the milk from milking to milking was made by a comparison of quarter differences when samples of milk were taken at consecutive milkings, morning and evening, for a period of three days. Chlorine and lactose were determined, and the

chlorine-lactose number was calculated. Data showing variation from milking to milking are shown in table 3.

The data as shown in table 3 represent one normal animal and three animals showing evidence of chronic mastitis. Number 475 was an animal definitely negative as shown by the various diagnostic tests. During the three-day period all samples were normal. In the case of cow number 469, quarters number 1 and 3 were definitely positive. All samples taken from these quarters were definitely abnormal as shown by the percentage chlorine and lactose as well as the chlorine-lactose number with the exception of samples taken from the third quarter on the last day. The second quarter at the morning milking also showed a positive difference for that time only. Animals number 486 and 490 are border-line cases. Cow number 486 was positive for chlorine in five out of six samples taken from quarter number 1, whereas the lactose was positive only for two samples, and the chlorine-lactose number was positive in four out of the six samples. Quarter number 2 was positive for one sample only for chlorine and chlorine-lactose number, and that was for the evening sample the last day. Cow number 490 gave milk from quarters 1 and 2 which was abnormal for chlorine. The chlorine-lactose number showed positive differences much of the time but the lactose did not show significant difference except for the three last samples taken from the second quarter. Quarter number 1 also showed a positive difference on the morning of the last day.

In table 4 are presented data for four cows which show the relationship between the development of chronic mastitis as determined by diagnostic tests and changes in the chlorine, lactose, chlorine-lactose number and casein number of the milk. The data for the four animals presented in table 4 are representative of the data as a whole.

In the case of cow 424 in table 4, only one abnormal sample was obtained from the third quarter and that was during the second month of lactation; nevertheless, this quarter was diagnosed suspicious for the first month and positive throughout the remainder of the lactation period. Quarters 1 and 2 were also diagnosed positive during the latter part of the period, but only the second quarter secreted abnormal milk. Cow 416 was diagnosed negative for mastitis during the latter part of the second lactation, but an abnormal sample was obtained from quarter number 1 during the eleventh month or the last month of the second lactation period. During the first three months of the third lactation all quarters were negative, but were either positive or suspicious for the fourth and fifth months. The second and fourth quarters secreted abnormal milk during the fifth month of lactation while the first and third quarters secreted normal milk even though mastitis was definitely diagnosed in these quarters.

Cow number 373 was diagnosed negative in all quarters until the eleventh month of the fourth lactation, at which time the fourth quarter

TABLE 4

Relationship of the development of chronic mastitis to changes in the chlorine, lactose, chlorine-lactose number and casein number of the milk

Cow #424, Second Lactation

	Number of quarters	Months of lactation							
		1	2	3	4	5	6	7	8
Diagnosis	1	–	–	S	S	S	+	+	+
	2	–	–	S	+	+	+	+	+
	3	S	+	+	+	+	+	+	+
	4	–	–	–	–	–	–	S	S
Chlorine	1	0.090	0.103	0.090	0.106	0.089	0.099	0.128	0.126
	2	0.091	0.107	0.095	0.103	0.097	0.125*	0.110	0.178*
	3	0.102	0.149*	0.107	0.107	0.108	0.104	0.112	0.120
	4	0.092	0.102	0.092	0.096	0.091	0.097	0.096	0.112
Lactose	1	4.70	4.75	5.20	5.25	5.10	5.15	4.80	4.85
	2	4.70	4.60	5.35	5.25	5.00	4.65*	4.75	3.50*
	3	4.50	3.65*	5.20	5.15	5.10	5.00	4.80	4.90
	4	4.65	4.55	5.75	5.40	5.45	5.25	5.05	5.15
Chlorine-lactose number	1	1.91	2.17	1.73	2.02	1.75	1.92	2.67	2.60
	2	1.94	2.33	1.78	1.96	1.94	2.69*	2.32	5.09*
	3	2.26	4.08*	2.06	2.08	2.18	2.08	2.33	2.45
	4	1.98	2.24	1.60	1.78	1.67	1.85	1.90	2.17
Casein number	1	77.8	77.6	78.1	77.3	75.3	76.9	76.5	79.2
	2	77.4	77.0	76.5	77.2	74.6	73.8*	75.0	59.7*
	3	76.0	71.7*	75.7	76.6	73.3	76.5	76.4	81.6
	4	77.8	76.8	78.1	78.3	75.8	77.3	77.5	77.6

TABLE 4.—(*Continued*)

Cow #416, Second and Third Lactations

	Number of quarters	Months of lactation									
		9	10	11	Dry	Dry	1	2	3	4	5
Diagnosis	1	–	–	–	....	....	–	–	–	+	+
	2	–	–	–	....	....	–	–	–	+	+
	3	–	–	–	....	....	–	–	–	+	S
	4	–	–	–	....	....	–	–	–	+	+
Chlorine	1	0.128	0.146	0.171*	....	....	0.093	0.112	0.099	0.110	0.112
	2	0.124	0.127	0.146	....	....	0.097	0.111	0.096	0.110	0.175*
	3	0.129	0.140	0.141	....	....	0.092	0.112	0.101	0.108	0.109
	4	0.127	0.140	0.141	....	....	0.091	0.111	0.099	0.109	0.175*
Lactose	1	....	....	3.65*	....	....	5.35	5.05	5.15	5.05	5.10
	2	....	....	4.15	....	....	5.25	5.00	5.20	5.05	3.45*
	3	....	....	4.00	....	....	5.25	5.00	5.15	5.15	5.10
	4	....	....	4.05	....	....	4.45*	5.00	5.05	5.15	3.60*
Chlorine-lactose number	1	....	....	4.68*	....	....	1.74	2.22	1.92	2.18	2.20
	2	....	....	3.52	....	....	1.85	2.22	1.85	2.18	5.07*
	3	....	....	3.53	....	....	1.75	2.24	1.96	2.10	2.14
	4	....	....	3.48	....	....	2.04	2.22	1.96	2.10	4.86*
Casein number	1	78.6	77.6	74.9*	....	....	77.4	75.5	75.7	76.8	74.1
	2	79.9	77.6	77.8	....	....	77.4	76.3	76.6	76.5	60.2*
	3	77.1	76.7	77.6	....	....	77.3	75.5	71.8*	76.4	78.1
	4	77.6	77.4	76.7	....	....	76.4	75.5	76.6	76.5	73.9*

TABLE 4.—(*Continued*)

Cow #373, Fourth and Fifth Lactations

	Number of quarters	Months of lactation									
		9	10	11	12	Dry	Dry	Dry	1	2	3
Diagnosis	1	–	–	–	S	......	......	......	S	S	S
	2	–	–	–	+	......	......	......	+	+	+
	3	–	–	–	S	......	......	......	S	S	S
	4	–	–	S	S	......	......	......	S	S	S
Chlorine	1	0.142	0.145	0.171*	0.174*	......	......	......	0.115	0.119	0.112
	2	0.139	0.146	0.161*	0.137	......	......	......	0.135*	0.155*	0.252*
	3	0.128	0.131	0.139	0.128	......	......	......	0.111	0.118	0.102
	4	0.138	0.146	0.153	0.146	......	......	......	0.125	0.124	0.125
Lactose	1	......	......	3.65*	3.70*	......	......	......	5.30	5.05	5.25
	2	......	......	3.75*	4.15	......	......	......	4.80*	4.45*	2.30*
	3	......	......	4.40	4.20	......	......	......	5.30	5.00	5.35
	4	......	......	4.65	4.00	......	......	......	5.05	4.90	5.00
Chlorine-lactose number	1	......	......	4.68*	4.70*	......	......	......	2.17	2.36	2.13
	2	......	......	4.29*	3.31	......	......	......	2.81*	3.48*	10.95*
	3	......	......	3.16	3.05	......	......	......	2.09	2.36	1.91
	4	......	......	3.29	3.65	......	......	......	2.47	2.53	2.50
Casein number	1	77.9	76.0	76.6	74.8*	......	......	......	77.9	78.8	77.8
	2	77.3	75.0	77.5	78.2	......	......	......	75.7	75.8*	66.7*
	3	78.7	78.0	79.1	78.4	......	......	......	77.9	79.7	78.5
	4	78.6	76.4	77.7	76.7	......	......	......	76.5	78.8	77.4

TABLE 4.—(*Continued*)

Cow #360, Fourth and Fifth Lactations

	Number of quarters	Months of lactation									
		9	10	Dry	Dry	1	2	3	4	5	6
Diagnosis	1	–	S	....	....	–	–	–	–	–	–
	2	–	S	....	....	S	S	S	S	+	+
	3	–	S	....	....	–	S	–	–	–	–
	4	–	S	....	....	–	S	+	+	+	S
Chlorine	1	0.150	0.166	....	....	0.106	0.118	0.128	0.119	0.120	0.123
	2	0.184*	0.218*	....	....	0.158*	0.131	0.128	0.157*	0.173*	0.184*
	3	0.143	0.173	....	....	0.108	0.113	0.126	0.119	0.127	0.120
	4	0.161	0.186*	....	....	0.112	0.131	0.147*	0.158*	0.139	0.212*
Lactose	1	....	....	....	....	5.35	5.30	5.07	5.10	5.10	5.05
	2	....	....	....	....	4.55*	5.10	4.73	4.45*	4.20*	4.00*
	3	....	....	....	....	5.30	5.40	5.05	5.20	5.05	5.10
	4	....	....	....	....	5.25	5.10	4.65*	4.55*	4.80	3.55*
Chlorine-lactose number	1	....	....	....	....	1.98	2.23	2.52	2.33	2.35	2.44
	2	....	....	....	....	3.47*	2.57	2.71	3.53*	4.12*	4.60*
	3	....	....	....	....	2.04	2.09	2.50	2.28	2.51	2.35
	4	....	....	....	....	2.13	2.57	3.16	3.47*	2.89	5.97*
Casein number	1	77.3	72.9	....	....	78.9	78.3	76.7	76.1	77.6	75.6
	2	74.3*	63.6*	....	....	74.5*	75.4*	73.3*	70.8*	69.3*	66.4*
	3	78.4	71.3	....	....	78.9	77.9	76.9	76.0	76.8	73.9
	4	76.0	68.7*	....	....	78.6	76.7	74.4	73.7	75.0	63.0*

* Milk abnormal composition.
\+ Positive for mastitis.
– Negative for mastitis.
S Suspicious for mastitis.

was diagnosed as suspicious. During the twelfth month the second quarter was diagnosed as positive and the others as suspicious. Quarter number 1 secreted abnormal milk high in chlorine and low in lactose during the eleventh and twelfth months of the fourth lactation. The second quarter secreted abnormal milk during the eleventh but apparently not during the last month of the lactation period. During the first months of the fifth lactation, the second quarter was diagnosed as positive while the other quarters were considered as suspicious. The first quarter did not continue to secrete abnormal milk after the intervening dry period between the fourth and fifth lactations which indicated improvement in the condition of the quarter during the dry period. However, the second quarter which was diagnosed as positive secreted abnormal milk high in chlorine and low in lactose from the first to the third month. The condition also became increasingly worse from the first to the third month.

Cow number 360 secreted milk of abnormal chemical composition from time to time in the second and fourth quarters during the latter part of the fourth lactation, and the first six months of the fifth. In the case of this animal there is some evidence that quarters may show some improvement and at times secrete milk of normal composition, while at other times the milk may be definitely abnormal. The second and fourth quarters were diagnosed as either suspicious or positive of mastitis.

DISCUSSION

From an examination of the data considerable evidence was obtained which indicates that certain micro-organisms generally considered to be the causal agents responsible for mastitis may be shed in the milk without any change in the chlorine, lactose or casein number of the milk at that particular time. The length of time which may elapse after the invasion of these organisms before any significant change in the chlorine, lactose and casein number is apparent, varies with different animals and with different quarters of the same animal. Many instances were observed where certain quarters were diagnosed positive during the latter part of the period but did not show a significant change in the chlorine, lactose or casein number during that particular lactation. This condition did not eliminate the possibility that eventually changes in these constituents of the milk might become apparent during the next lactation. However, certain individuals shedding these organisms during the latter part of lactation began the next lactation without any indications of mastitis.

It is evident that the biochemical tests used will not indicate quarters showing incipient stages of chronic mastitis.

Significant changes in the chlorine, lactose or casein number of the foremilk based upon quarter differences were almost universally associated with mastitis, but on the other hand many quarters were diagnosed as positive

for mastitis which did not show significant difference between diseased and normal quarters at that particular time. Apparently there must be a definite change of the mammary tissue before the composition of the milk of the individual quarter is affected. Further evidence for this statement might be obtained by histological examinations of udders which are known to have secreted milk of abnormal composition.

The data also indicate that changes in the percentage of chlorine, lactose, chlorine-lactose number and casein number take place simultaneously. There were a few exceptions, but the possibility of analytical error was not entirely eliminated. Since some of these constituents may be derived directly from the blood by diffusion through the cell membranes, while others must be synthesized in the mammary gland, it appears as though in mastitis there must be a change in the synthetic and secretory mechanism of milk secretion as well as an increase in the permeability of the cell membranes.

For detecting chemical changes in the milk due to or associated with mastitis, the determination of the casein number was no more desirable than a determination of either the chlorine or the lactose. The determination of the chlorine and lactose with the calculation of the chlorine-lactose number is to be preferred over either chlorine or lactose alone.

Changes in the percentage of chlorine and lactose with corresponding changes in the chlorine-lactose number based upon quarter differences in the same udder can be recommended as an indirect biochemical method for detecting chronic mastitis which has developed to the stage of causing changes in the composition of the milk of affected quarters.

Diagnosis of chronic mastitis by changes in the percentage chlorine, lactose, chlorine-lactose number or casein number due to or associated with mastitis and based upon quarter differences instead of a set standard, practically eliminates difficulty due to changes in the chemical composition of the milk from time to time as well as changes associated with age and advanced stages of lactation and certain peculiarities of the individual animals.

SUMMARY AND CONCLUSIONS

In this study approximately 250 udder examinations on 40 purebred Holstein cows were made. The percentages of chlorine, lactose, total nitrogen and non-casein nitrogen were determined on samples of fore-milk from individual quarters, and from these the chlorine-lactose number and the casein number were calculated.

Physical examination and the following diagnostic tests on samples of fore-milk were made in conjunction with the chemical studies: strip cup, brom thymol blue, Hotis test, chlorine (colorimetric), microscopic examination of incubated milk, leucocyte count, and blood agar plate.

The fore-milk from individual quarters free from mastitis was found to

contain from 0.090 to 0.224 with an average of 0.124 per cent chlorine, and from 3.30 to 5.40 with an average of 4.788 per cent for lactose. The chlorine-lactose number varied from 1.62 to 6.79 with an average of 2.614, while the casein number varied from 69.4 to 84.3 with an average of 77.57.

Milk secreted by quarters in the advanced stages of mastitis contained a higher percentage of chlorine and a lower percentage of lactose than milk from normal quarters. The chlorine-lactose number was also higher and the casein number lower in milk from quarters with mastitis than from normal quarters.

The detection of changes in the chlorine, lactose or casein number of milk due to or associated with mastitis based upon quarter differences practically eliminates difficulty due to changes in the chemical composition of the milk from time to time as well as changes associated with age, advanced stages of lactation, and certain peculiarities of the individual animals.

The mean difference between quarters in normal udders free from mastitis was for chlorine content 0.0065 per cent, lactose 0.115 per cent, chlorine-lactose number 0.184, and casein number 0.999.

In order to detect affected quarters by changes in the chemical composition of the milk from individual quarters, the quarter with the lowest per cent chlorine and chlorine-lactose number, or the highest per cent lactose and casein number was considered normal. For a significant difference between normal quarters and affected quarters there must be a difference of at least 0.02 per cent chlorine, 0.36 per cent lactose, 0.60 for chlorine-lactose number, and 2.90 for casein number when samples of six to eight ounces of milk were used.

Individual quarters usually showed bacteriological changes in the fore-milk before a change in the chemical composition of the milk was apparent.

Indirect biochemical tests such as the percentage of chlorine, the percentage of lactose, the chlorine-lactose number or the casein number when based upon quarter differences in the same udder can be recommended for the diagnosis of chronic mastitis. However, these tests will not indicate quarters which show chronic mastitis in the incipient stages.

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THE EFFECT OF COCOA UPON THE DIGESTIBILITY OF MILK PROTEINS

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INTRODUCTION

Before any substance is added to milk, one should be certain that it will not destroy some of the well-known nutritive properties of plain milk. No doubt the increase in milk consumption, especially by children, as a result of adding chocolate flavoring would be desirable, provided it did not injure the nutritional properties of the milk.

Mueller and Ritchie (1) found that the addition of 4 per cent by weight of cocoa to plain milk, lowered the gain in weight of rats by approximately 17 per cent, even though the amount of milk consumed was the same as when a plain whole milk diet was fed. It was thought that this observed decrease in rate of growth might be due in part to a decrease in the digestibility of the milk proteins as a result of adding cocoa.

Ulrich (2) found that cacao beans may contain approximately 8 per cent by weight of cacao red. In its reactions with proteins, cacao red much resembles tannin. Whymper (3) states that tannin decreases the solubility of milk solids.

Neumann (4, 5) studied the digestibility of cocoa, using himself as a subject for 86 days, and reported that the addition of cocoa to other articles of food seemed to reduce the total amount of nitrogen absorbed. He also found that the amount of fat present in cocoa affected the absorption of nitrogen, a reduction in fat lowering the assimilation of nitrogen.

Aplin and Ellenberger (6), working with cows, found that the addition of two pounds of cocoa meal (a by-product of the chocolate industry) to the daily amount of food consumed by the cows reduced the coefficient of digestibility of the crude protein from 55 per cent for the cocoa-free food to 50 per cent for the diet containing cocoa.

Mitchell *et al.* (7), studying the digestibility of cocoa and milk proteins, concluded that no marked supplementary relation exists between the nitrogenous compounds of milk and of cocoa. In their studies 50 per cent of the nitrogen in the food used came from the cocoa and the other 50 per cent from the milk.

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OBJECT OF STUDY

This study was undertaken with the hope of demonstrating by animal feeding experiments whether or not the addition of cocoa to whole milk powder (in amounts equivalent to approximately 4 per cent by weight on a fluid milk basis) had any adverse effect upon the digestibility of the milk proteins. The effect of both the Dutch-process and the American-process cocoa as well as the effect of added cocoa fat was studied.

It was also hoped that the data obtained in this study would aid in explaining the cause for the retarded rate of growth of white rats, as reported by Mueller and Ritchie (1).

EXPERIMENTAL

Determining Digestibility of Proteins in Milk and Milk Plus Cocoa. In order to study the effect of cocoa upon the digestibility of the milk proteins, the following determinations had to be made: 1, Digestibility of proteins in milk alone; 2, Digestibility of proteins in milk plus cocoa; 3, Digestibility of the cocoa proteins in the absence of milk proteins; and 4, Metabolic nitrogen. Twenty-four white rats from the same colony and nine weeks old were used for the entire study.

In the first study the rats were divided into four groups, each group consisting of six rats, three males and three females, except group IV, which consisted of two females and four males. The groups were arranged so that the average weight was approximately the same.

The feed formulas for the four groups are given in table 1, which also gives the total nitrogen as determined by the Kjeldahl method. It should be noted that these diets are equivalent on a fluid milk basis to approximately 3.6 per cent cocoa, 6.3 per cent cane sugar and 13 per cent milk solids. Whole milk powder was used in this study instead of fluid milk, so that all of the feed for a trial could be prepared at the same time. This insured that the composition of the feed would not vary from day to day as might be the case if fresh fluid milk were used. Also, milk powder from the same batch could be used for a number of trials, thus insuring that the milk proteins used in all trials had received the same previous treatment. Another advantage of the dry feed is that the cocoa powder will stay evenly distributed and will not settle as it does when added to fluid milk. A third advantage is that the uneaten food could be determined by weight, whereas the amount of fluid milk left cannot be determined so accurately, due to losses by evaporation.

Group I is the control group, which was fed the basal diet consisting of whole milk powder, sugar, and salt mixture (Osborne and Mendel). Group II was fed a diet containing the same constituents as Group I, but containing 15.8 per cent Dutch-process cocoa. The diet for Group III was the same as for Group II except that American-process cocoa was used instead of the Dutch-process cocoa. Group IV's diet contained the same constituents as

TABLE 1

Feed formula used in determining digestibility of milk and milk plus cocoa proteins

Ingredient	Group I	Group II	Group III	Group IV
	%	%	%	%
Whole milk powder	62.9	52.6	52.6	48.7
Cane sugar	33.1	27.6	27.6	25.6
Cocoa	....	15.8 (D)	15.8 (A)	14.6 (A)
Cocoa fat	......	....	....	7.1
Osborne and Mendel salt mixture	4.0	4.0	4.0	4.0
Cocoa on fluid milk basis	0.0	3.6	3.6	3.5
Total nitrogen	2.45	2.49	2.50	2.48

(A) Indicates American-process.
(D) Indicates Dutch-process.

Group III's, except that pure cocoa fat was added, in order to raise the cocoa fat content to the same level as if chocolate liquor had been used.

The rats were individually caged and were fed the diet ad libitum for a period of 18 days to thoroughly accustom them to it. During this period, various preliminary tests were carried out. The feed was placed in Fisher porcelain cups of 50 cc. capacity which were in turn placed in large metal feed cups with covers. The purpose of the metal feed cup was to catch the feed which was spilled from the porcelain cups by the animals. The rats had water before them at all times.

The rats were fed 10 grams of feed daily during the test period of three days, as preliminary trials showed that they consumed about 10 grams of feed daily. The first feeding of 10 grams was marked with carmine. Paper towels were placed under the grid floor of the cages to catch the feces, and the feces marked with carmine (red) were collected. Ten more grams of feed were fed on both the second and third days. On the fourth day, the amount of feed left, including that spilled into the metal feed cups was weighed and the total amount of feed consumed was determined. The cups were cleaned, then feed marked with charcoal was given the rats and the feces were collected until the marked feces came through (black). Each rat's feces were collected daily and stored separately in a refrigerator (0° F.). The feces from each rat were weighed and ground in a mortar. A 2-gram sample of the ground feces was analyzed for total nitrogen by the Kjeldahl method, and the total amount of nitrogen in the feces calculated. The animals were weighed on the first and fourth days of the test period. The results of this experiment are shown in table 4.

Determining Digestibility of Proteins in Cocoa. In this experiment the feeds for Groups II, III, and IV were made up to contain approximately the same amounts of cocoa and cocoa fat on a percentage basis as feeds described in the preceding experiment. The same rats were used as before and were kept in the same groups. The only source of protein in the diet was the cocoa, while in the previous experiment the protein was furnished by both

cocoa and milk powder except in Group I, which contained only milk proteins. The composition of the feeds is given in table 2, which also gives the amount of nitrogen. The rats were fed ad libitum for three days previous to the test. During the test period of three days the rats were fed 10 grams of feed daily. The food was marked, the feces collected and stored, and the total nitrogen consumed and excreted was determined as before. The animals were weighed on the first and fourth days of the test period. Results of this study are shown in table 5.

TABLE 2

Feed formula used in determining digestibility of cocoa proteins

Ingredient	Group II	Group III	Group IV
	%	%	%
Corn starch	68.5	68.5	62.4
Osborne and Mendel salt mixture	4.0	4.0	4.0
Butter fat	9.0	9.0	9.0
Cod liver oil	2.0	2.0	2.0
Cocoa	16.5 (D)	16.5 (A)	15.2 (A)
Cocoa fat	......	......	7.4
Total nitrogen	0.74	0.74	0.60

(D) Indicates Dutch-process.
(A) Indicates American-process.

Determining the Metabolic Nitrogen. The fecal metabolic nitrogen is the small amount of nitrogen that is found in the feces when the animal is fed a low-protein diet. It is composed of epithelial cells, bacteria, mucus and the residues of the bile and digestive juices. Theoretically, during the determination of the fecal metabolic nitrogen, the rats should be fed on a non-protein diet. However, the work of various investigators has shown that the rats must be fed at least 4 per cent of protein or they will lose weight very rapidly and die within a short time. In his earlier work Mitchell (8) fed a protein-free diet while determining metabolic nitrogen, but in later experiments (9, 10), a diet containing small amounts of protein was used. Kon (11) and Schneider (12) also used a low-protein diet in determining metabolic nitrogen.

TABLE 3

Feed formula used in determining metabolic nitrogen

Ingredient	Groups I, II, III	Group IV
	%	%
Corn starch	80.0	74.5
Osborne and Mendel salt mixture	4.0	3.7
Butter fat	9.0	8.4
Cod liver oil	2.0	1.9
Casein	5.0	4.6
Cocoa fat	......	6.9
Total nitrogen	0.91	0.79

The composition and total nitrogen of the diets used for determining metabolic nitrogen are given in table 3. The rats were fed the food for three days before the test period. During the test period which lasted three days, the rats were fed 10 grams of feed daily. The food was marked, amount consumed determined, feces were collected, and the total nitrogen excreted was determined as before. All animals were weighed on the first and fourth days of the test period. The results are shown in table 6.

In determining the value for the fecal metabolic nitrogen, to be used in

TABLE 4

Digestibility of the proteins in the various diets

Rat no.	Initial weight	Final weight	Food intake	Nitrogen intake	Nitrogen in feces	Metabolic nitrogen in feces	Food nitrogen in feces	Nitrogen digested
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
Group I—62.9 per cent milk powder								
1	140	141	23.5	.576	.135	.046	.089	84.5
2	150	146	30.0	.737	.170	.055	.115	84.4
3	148	153	28.0	.687	.180	.054	.126	81.7
4	171	171	29.8	.731	.167	.068	.099	86.5
5	173	174	28.2	.692	.144	.055	.089	87.1
6	180	178	29.9	.733	.149	.059	.090	87.7
								Av. 85.3
Group II—52.6 per cent milk powder and 15.8 per cent Dutch-process cocoa								
7	130	128	29.6	.737	.303	.057	.246	66.7
8	131	130	29.7	.740	.251	.063	.194	73.6
9	138	138	30.0	.747	.301	.060	.241	67.9
10	146	154	30.0	.747	.238	.054	.184	76.7
11	152	157	30.0	.747	.335	.056	.279	62.7
12	157	155	29.9	.745	.316	.059	.257	65.5
								Av. 68.9
Group III—52.6 per cent milk powder and 15.8 per cent American-process cocoa								
13	144	140	28.3	.706	.283	.054	.229	67.7
14	155	152	29.8	.744	.238	.057	.181	75.7
15	170	166	29.9	.746	.215	.059	.156	79.1
16	161	160	30.0	.749	.332	.060	.272	63.6
17	184	180	30.0	.749	.259	.058	.201	73.0
18	192	175	30.0	.749	.302	.060	.242	67.8
								Av. 71.2
Group IV—48.7 per cent milk powder and 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat								
19	116	116	30.0	.745	.221	.059	.162	78.3
20	166	163	29.9	.742	.272	.062	.210	70.4
21	158	156	30.0	.745	.264	.059	.205	72.6
22	154	147	30.0	.745	.280	.063	.217	70.9
23	160	165	29.9	.742	.288	.061	.227	66.8
24	171	167	30.0	.745	.300	.059	.241	67.7
								Av. 71.1

calculating the digestibility of the food proteins, the metabolic nitrogen was corrected by multiplying the metabolic nitrogen per gram of low-protein food by the number of grams of food consumed during the trial under consideration. This correction is necessary since Mitchell (10), and later Schneider (12) have shown that the metabolic nitrogen is proportional to the amount of food consumed.

TABLE 5

Digestibility of proteins in cocoa

Rat no.	Initial weight	Final weight	Food intake	Nitrogen intake	Nitrogen in feces	Metabolic nitrogen in feces	Food nitrogen in feces	Nitrogen digested
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
Group II—16.5 per cent Dutch-process cocoa								
7	120	113	26.2	.193	.156	.050	.106	45.1
8	131	118	29.7	.220	.206	.063	.143	35.0
9	125	120	29.6	.219	.177	.060	.117	46.9
10	144	137	22.6	.167	.154	.042	.112	32.9
11	150	138	28.1	.207	.195	.053	.142	31.3
12	144	140	28.0	.207	.185	.055	.130	37.2
								Av. 38.1
Group III—16.5 per cent American-process cocoa								
13	147	128	28.0	.207	.185	.054	.131	37.0
14	143	133	28.5	.211	.176	.053	.123	41.5
15	161	149	29.1	.215	.150	.059	.109	58.1
16	148	142	26.1	.193	.159	.052	.107	44.9
17	175	165	29.5	.218	.179	.058	.121	44.7
18	180	163	26.9	.199	.172	.054	.118	41.0
								Av. 44.5
Group IV—15.2 per cent American-process cocoa and 7.4 per cent cocoa fat								
19	114	107	23.5	.141	.128	.047	.081	42.6
20	155	146	29.5	.177	.152	.062	.090	49.0
21	153	140	27.3	.163	.169	.053	.116	29.2
22	141	135	24.7	.148	.134	.052	.082	45.0
23	153	143	28.7	.172	.170	.059	.111	35.8
24	160	148	24.9	.149	.131	.049	.082	44.8
								Av. 41.1

DISCUSSION OF RESULTS

The effect of cocoa on the digestibility of the milk proteins is shown in table 7. The following formula was used in making the calculations:

$$D = \frac{(C - B)(X) + B(A)}{C}$$

Solving for X:

$$X = \frac{C(D) - B(A)}{(C - B)}$$

Where X = digestibility of milk proteins
D = digestibility of mixture of cocoa proteins and milk proteins
A = digestibility of cocoa proteins
B = total nitrogen in feed from cocoa
C = total nitrogen in feed
C − B = total nitrogen in feed from milk

The analysis of the American- and Dutch-process cocoas for total nitrogen gave values of 0.0382 and 0.0367 grams per gram of cocoa powder respectively.

A value of 85.3 per cent was obtained for the digestibility of milk proteins in whole milk powder. This value is somewhat lower than the average which has been reported in the literature by various investigators. Fairbanks and Mitchell (13), and Nevens and Shaw (14) have shown that the

TABLE 6

Determination of metabolic nitrogen

Rat no.	Initial weight	Final weight	Food intake	Nitrogen in feces (metabolic)
	grams	*grams*	*grams*	*grams*
Group I				
1	137	135	28.2	.053
2	148	146	29.8	.055
3	152	148	29.0	.054
4	171	166	25.0	.057
5	171	166	23.4	.047
6	182	178	28.2	.059
Group II				
7	119	119	30.0	.059
8	119	116	25.0	.053
9	113	112	30.0	.060
10	136	131	14.0	.025
11	144	141	21.0	.039
12	142	135	24.0	.047
Group III				
13	134	129	23.0	.044
14	145	139	28.0	.053
15	154	145	20.0	.040
16	139	134	30.0	.060
17	163	159	25.0	.048
18	164	158	30.0	.060
Group IV				
19	103	102	23.0	.045
20	150	148	30.0	.062
21	146	145	27.0	.053
22	135	134	30.0	.063
23	142	147	29.0	.059
24	159	141	23.0	.045

digestibility of the milk proteins is lowered by the heat treatment to which the milk is subjected during the drying process. Therefore, considerable variation in the digestibility of proteins in various milk powders may be expected. Mitchell (8) found that there is a slight decrease in the digestibility of milk proteins as the protein level is increased from 5 to 10 per cent. In the present study the protein level fed was 15 per cent, which may be a contributing factor for the somewhat low value of 85.3 per cent.

TABLE 7

Effect of cocoa on the digestibility of milk proteins

Kind of diet	Average digestibility of milk proteins	Difference when compared with plain milk	Decrease when compared with plain milk
	per cent probable error		*per cent*
Basal ration (whole milk powder, cane sugar and salt mixture)	85.3 ± 0.56	....	....
Basal ration including 15.8 per cent Dutch-process cocoa	78.6 ± 1.90	−6.7	7.85
Basal ration including 15.8 per cent American-process cocoa	80.1 ± 1.63	−5.2	6.09
Basal ration including 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat	80.3 ± 1.46	−5.0	5.86

The calculated digestibility (see table 7) of the milk proteins in diets containing approximately 15.8 per cent of cocoa, show that the Dutch-process cocoa lowered the percentage digestibility from 85.3 (± .56) to 78.6 (± 1.90), while the American-process cocoa lowered the percentage digestibility from 85.3 (± .56) to 80.1 (± 1.63). Thus no significant difference was noted for the two kinds of cocoa. Also the inclusion of 7.1 per cent by weight of cocoa fat to the milk powder–American-process cocoa ration, did not alter to any marked extent the digestibility of the milk proteins. When the same method of calculation as used in this study is applied to the results obtained by Mitchell (7) the values obtained show that the percentage digestibility of the milk proteins is lowered from 95 to 88 when Dutch-process cocoa was added to the diet.

The results in this study that cocoa decreases the digestibility of the milk proteins is in agreement with Neumann's (4, 5) report that the addition of cocoa to other articles of food seemed to reduce the total amount of nitrogen absorbed. However, it should be mentioned that Neumann's experiments were performed on a human subject, whereas white rats were used in this study.

Although the observed decrease in digestibility of milk proteins when cocoa was added is not marked, no doubt it is of some significance in explaining the results by Mueller and Ritchie (1). These investigators found that

the addition to a whole milk powder ration of 17.8 per cent by weight of Dutch-process cocoa, equivalent to 4 per cent by weight on fluid milk basis, lowered the rate of weight gain in rats by 17 per cent, even though the amount of milk powder consumed was the same as when a plain whole milk powder diet was fed.

It will be noted in table 7 that the probable error for the digestibility of milk proteins is greater when cocoa is added to whole milk powder as compared to whole milk powder alone. This is to be expected since the determination of the digestibility of the milk proteins in the presence of cocoa is a more complicated procedure than when milk powder is the only source for the proteins. Furthermore, it is readily conceivable that the decrease in palatability of the ration, as a result of including 15.8 per cent by weight of cocoa may be a contributing factor in increasing the probable error.

The digestibility of the proteins in cocoa is of little practical significance in chocolate milk, due to the small amount of cocoa used. However, it was necessary to determine the digestibility of the cocoa proteins in this study, in order to calculate the digestibility of the milk proteins when cocoa was added to the milk powder. The value obtained for the digestibility of the protein from Dutch-process cocoa was 38.1 per cent, and agrees with that obtained by Mitchell *et al.* (7). A value of 44.5 per cent was obtained for the digestibility of the protein in American-process cocoa, and this value was lowered to 41.1 per cent when 7.4 per cent by weight of cocoa fat was included in the ration.

SUMMARY AND CONCLUSIONS

Whole milk powder plus a commercial brand of Dutch-process cocoa and whole milk powder plus a commercial brand of American-process cocoa, with and without additional cocoa fat, were fed in comparison with whole milk powder in feeding trials with albino rats. The digestibility of the milk proteins was studied.

The rats were able to digest approximately 85, 69, 71, and 71 per cent of the food proteins, when rations containing 62.9 per cent milk powder, 52.6 per cent milk powder and 15.8 per cent Dutch-process cocoa, 52.6 per cent milk powder and 15.8 per cent American-process cocoa, 48.7 per cent milk powder and 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat were fed, respectively. Subjecting these results to mathematical analysis revealed that the digestibility of milk proteins (85.3 per cent) was reduced 7.8 and 6.0 per cent when the ration contained 15.8 per cent Dutch, and 15.8 per cent American-process cocoa, respectively. The inclusion of 7.1 per cent of cocoa fat to the American-process cocoa–milk rations reduced the digestibility of the milk proteins by 5.8 per cent.

The proteins of the American-process cocoa were more completely digested (44.5 per cent) than those of Dutch-process (38.1 per cent), when the

ration contained 16.5 per cent of cocoa and cocoa was the only source of protein in the diet. The digestibility of the proteins in the American-process cocoa was found to be only 41.1 per cent when 7.4 per cent by weight of cocoa fat was included in the ration.

On the basis that the addition of cocoa to whole milk powder (in quantity equivalent to approximately 3.6 per cent by weight on a fluid milk basis) does not greatly reduce the digestibility of the milk proteins, we may conclude that the amount of cocoa in average commercial chocolate milk (approximately 1 per cent by weight) has no significant adverse effect upon the digestibility of the milk proteins.

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CARE OF MILKING MACHINES[1]

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In a recent paper by Mallman, Bryan and Begeman (3) it is stated: "There is a dearth of information on methods of cleaning milking machines. Most publications place emphasis on the sanitation. The most common practice in cleaning consists of flushing machines with cold water after each milking and then placing the tubes on the racks for either alkali or chlorine treatment. Under these conditions machines are washed once a week with hot water and a detergent. This procedure is very poor operating practice." Several of these statements should not be allowed to pass unquestioned. That "there is a dearth of information on methods of cleaning milking machines" is difficult to reconcile with the extensive literature on the subject. The assertion that flushing tubes with cold water, then filling with alkali solution "is very poor operating practice" should be supported by definite evidence since it is at variance with the results reported by several investigators (1, 2, 4, 5). Finally, the inference that tubes are not considered properly washed unless hot detergent solution is used after each milking also appears to run counter to the facts.

Until 1932 the need for a suction rinse with hot detergent solution was generally admitted. However, as the above-mentioned authors admit, this "is seldom carried out in practice, and even where it is used the method is not carried out with sufficient thoroughness to render the equipment free from milk wastes and lime deposits." In 1932, however, it was shown (2) that a cold lye solution, acting during the long interval between milkings, maintains the rubber tubing, etc., in as clean condition as does a brief rinse with hot detergent solution. The lye solution should therefore be regarded as a detergent as well as a germicide. On the other hand, where the tubes are rinsed with cold water, then filled with chlorine solution (which has no appreciable detergent action) the results are much less satisfactory (2). Presumably the objectionable conditions referred to by Mallmann *et al.* were due to the use of chlorine in place of lye, although their blanket condemnation of the cold water rinse method applies to both lye and chlorine solutions.

The efficiency of the lye solution method, following a cold suction rinse, in maintaining milker tubes in satisfactory bacteriological condition has been demonstrated by Johns (2), by Hastings and Werner (1), and by Parfitt (4). Rogers and Evans (5) have likewise shown that immersion between

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milkings in a cold solution of trisodium phosphate maintains the tubes in a satisfactory state. Further evidence of the effectiveness of the lye solution method is presented in table 1, based upon routine counts obtained on the milk from the Central Experimental Farm Dairy between January 1, 1938, and December 31, 1939. (Filling the tubes with weak lye solution following a suction rinse with cold water, has been standard practice since August, 1930). The counts here summarized represent routine plate counts on samples of mixed night's and morning's milk taken from the pasteurizing vat before heating, hence contamination from various sources other than the milking machines is included. It will be observed that 80.7 per cent of the counts were below 5,000 per ml. and 94.2 per cent below 10,000 per ml. The highest count recorded was 29,000.

TABLE 1

Distribution of plate counts on routine raw milk samples
(C. E. F. dairy, 1938–1939)

	Bacteria counts*							Total
	< 2,500	2,501–5,000	5,001–7,500	7,501–10,000	10,001–15,000	15,001–25,000	25,001–50,000	
Number	81	87	18	10	6	5	1	208
Percentage	38.9	41.8	8.7	4.8	2.9	2.4	0.5	100

* Standard methods of A.P.H.A. followed, except that tryptone-glucose-skim milk agar was introduced January 1, 1939, instead of July 1.

TABLE 2

Monthly average (logarithmic) counts on routine samples of raw and pasteurized milk
(C. E. F. dairy)

Month	No. of samples	Raw milk			Pasteurized milk
		Plate count	Coliform bacteria* in		Plate count
			0.1 ml.	0.01 ml.	
1933			%	%	
September	7	5,430	71.5	35.7	97
October	9	19,240	37.7	17.0	142
November†	7	10,530	57.1	28.6	147
December	5	6,850	70.0	20.0	63
1934					
January	8	8,350	12.5	0.0	58
February	7	6,950	0.0	0.0	30
March	8	5,370	28.5	0.0	38
April	7	17,200	14.3	0.0	33
May†	9	6,220	66.7	22.2	23
June	10	10,330	75.0	40.0	36
July	8	6,960	81.0	31.0	17
August	5	13,440	100.0	100.0	16

* Percentage of tubes of brilliant green lactose bile broth showing gas.
† Between Nov. 27 and May 4, two units received no rinse of any sort, going direct from cow to solution rack where they were filled with 0.5% lye solution.

Still more striking evidence of the detergent action of weak lye solution was obtained some years ago. Due to a misunderstanding, two milker units failed to receive even a cold water rinse at the barn, being placed directly on the solution rack and filled with 0.5 per cent lye solution. The usual weekly disassembling and brushing with hot detergent was also omitted. This continued from November 27, 1933, to May 4, 1934, at which time it was necessary to replace the liners. Data from routine analyses of both raw and pasteurized milk during the period September, 1933, to August, 1934, are summarized in table 2. At the conclusion of these studies, some calcium phosphate from the milk residue was found deposited in the liners and tubes. This condition was not reflected in higher bacteria counts in either raw or pasteurized milks, despite the claim (3) that no chemical disinfection will function when the surface to be disinfected is covered with such deposits. The omission of all rinsing to remove the residual milk is definitely not to be recommended, but the results show very clearly how effective the lye solution is, both as a detergent and as a germicide. This is confirmed by the results from a number of farms where this method is employed.

Since all available evidence indicates that milker tubes can be kept in good sanitary condition by filling with lye solution following a cold water rinse, it seems difficult to justify the recommendation that machines be given an additional rinse with hot detergent solution. While the additional rinse will admittedly lower the bacteria count slightly (2, 4) this extra step would only be justified where measures were being taken to reduce bacterial contamination to the absolute minimum. The level of bacteria counts reported by Mallmann *et al.* (3) (geometrical mean of 172,000 per ml.) for milk drawn by machines subjected to a "satisfactory" method of cleaning suggests that the milk supplies which they investigated were scarcely in this category.

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THE pH VALUES OF THE INGESTA OF THE RUMEN OF SLAUGHTERED ANIMALS[1]

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In our work on bloat in bovines, we visited several packing plants to obtain samples of rumen gas. After the gas had been obtained the rumen was slit open, and a four-ounce sample of the rumen contents was taken. Samples of ingesta were also obtained from animals when no gas samples had previously been taken. No effort was made to obtain the ingesta in any particular part of the rumen. The sample consisted largely of the juice. When the juice was difficult to obtain by dipping, a quantity of ingesta was squeezed in the hand.

The juice was taken to the laboratory, where the pH was determined on a Coleman's electric potentiometer. In several instances the pH was determined the afternoon of the same day on which the ingesta samples were collected. In other cases the pH values were not determined until approximately 20 hours after the samples were taken.

The animals from which the samples were taken had been purchased for slaughter. Many of them were yard fed, while others were grass fed before being received at the plant. As is customary when animals are delivered to the yards for slaughter, they are fed a limited quantity of roughage, usually a non-legume such as prairie hay.

Whenever it was possible to identify the type of feed or grain in the ingesta, this was done. Obviously it was not possible to identify all the grains, or any finely ground feed.

TABLE 1

	No. of samples	Time elapsed before pH was determined	Range	Average
		hr.	*pH*	*pH*
Dec. 1, 1939	104	22	7.59–6.41	6.87
" 27, 1939	55	22	7.40–6.41	6.98
" 29, 1939	51	5	7.20–5.70	6.65
" 30, 1939	48	5	7.13–6.16	6.76
Jan. 3, 1940	37	22	7.45–5.90	6.99
" 4, 1940	55	7	7.78–5.52	6.89
June 6, 1940	8	8	7.56–6.89	7.07
" 6, 1940	42	8	7.39–5.97	6.81
" 18, 1940	41	7	7.45–6.08	6.88
July 3, 1940	32	45	7.55–5.56	6.89
	473			Av. 6.859

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TABLE 2

Average pH values of ingesta held under room temperatures for a given period following collection

Sample no.	Number of samples	5 hr.	7 hr.	8 hr.	22 hr.	30 hr.	45 hr.	70 hr.	95 hr.	120 hr.	150 hr.	168 hr.	235 hr.
		pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
1	104				6.87							7.57	
2	55				6.98					7.30			
3	51	6.65										7.07	
4	48	6.76										6.95	
5	37				6.99							8.29	
6	55		6.89			6.97		7.22	7.49				
7	8			7.07	7.25		7.31		7.77		8.07		
8	42			6.81	7.04		7.13		7.64		7.92		
9	41		6.88		6.86		7.00		7.47		7.45	7.51	
10	32						6.89			7.22		7.70	7.96
	473	6.70	6.88	6.85	6.95	6.97	7.02	7.22	7.54	7.27	7.72	7.49	7.96

RESULTS

Samples of ingesta were collected from slaughtered animals on the following dates, and the pH values determined at the stipulated time. (See table 1.)

The samples of ingesta were held at room temperature for various lengths of time and the pH values determined to note what changes if any in the pH followed the respective holding periods. The data in table 2 indicate the results.

DISCUSSION

The samples of ingesta were obtained from cows, steers and a few from aged bulls. Some of these were of beef strains, others of dairy. Some were choice beef animals, others were canners. No significant differences in pH values were obtained in these various animals.

The animals on which notations on the feed in the rumen at time of slaughter were made, did not reveal data from which definite conclusions on the effect of feed on pH can be drawn. It did seem, however, that those animals which showed hay or straw only in the rumen had a somewhat higher pH value. Yet in many instances the pH values were no higher than when grain was present with the roughage. In sample No. 7 eight samples of ingesta were obtained from cows whose ingesta indicated they had been fed on green grass. These were more alkaline than the average of the other samples.

The pH values on the 473 samples averaged 6.859. In checking the pH values of the groups of samples collected at different times of the year and therefore from animals under different feeding conditions no appreciable variation is noted. Monroe and Perkins (1) reported higher acid on rumen ingesta from cows on pasture than when roughages, corn, and A.I.V. silage were fed.

They also found when samples of ingesta were taken at two hour intervals, those taken prior to the morning feeding were the most alkaline. After feeding, the ingesta became more acid, with a trend toward greater alkalinity after several hours following the feeding period.

The same authors found the ingesta of animals which were slaughtered 12 to 18 hours after the last feeding to have a pH value of 7.34. This is somewhat more alkaline than the average of the samples reported herein. Undoubtedly many of the samples reported in our work were taken from cows which had not eaten any roughage for at least 12 hours before slaughter as it is customary to withhold feed from animals about 12 hours before slaughtering.

The data in table 2 indicate very definitely that the ingesta increased in alkalinity when it was allowed to remain in the laboratory at room temperature. In sample numbers 7, 8 and 9, the pH determinations covered a period of 6 to 7 days, with an appreciable increase in alkalinity.

The work of Monroe and Perkins (1) indicated an appreciable increase in acidity when the ingesta were incubated for 8 hours at 100° F.

No explanation is suggested for the ingesta becoming alkaline in the trials reported herein, on standing. If acid producing organisms were present in the ingesta in appreciable numbers the opposite would be expected. However, the data on a fairly large number of samples indicated that the samples of ingesta on standing were alkaline.

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TABLES AND NOMOGRAPH FOR SHARP AND HART'S EQUATION FOR THE CALCULATION OF TOTAL SOLIDS IN MILK

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It has long been known that the previous temperature history and manner of treatment received by milk has an influence upon its specific gravity as measured at 15° C. An important contribution towards a method to eliminate this influence was made by Bakke and Honegger (1). Their work was extended and corroborated by Hoyt, Lampert *et al.* (2). Lampert (3) also made a general review of this and other work pertaining to the use of the lactometer.

In a detailed study of the subject, Sharp and Hart (4) recommend that the specific gravity of milk be measured at 30° C., the milk previously having been warmed to 45° C. for one-half minute, thus insuring that the determination is made while the fat is in the liquid state. The following equation was evolved for the calculation of total solids from the fat content and specific gravity:

$$\text{Total solids} = 1.2537 \text{ fat} + 0.2680 \frac{\text{lactometer}}{\text{sp.gr. of milk}}.$$

In 421 comparisons the deviation between calculated and gravimetric results did not exceed 0.30 per cent. This variation is comparable to that which may result when the ordinary Babcock procedure is used to calculate

TABLE 1

Fat factors for total solids in milk according to Sharp and Hart's equation

Per cent fat	Factor	Per cent fat	Factor	Per cent fat	Factor
0.1	0.1254	2.1	2.633	4.1	5.140
0.2	0.2507	2.2	2.758	4.2	5.266
0.3	0.3761	2.3	2.884	4.3	5.391
0.4	0.5015	2.4	3.009	4.4	5.516
0.5	0.6269	2.5	3.134	4.5	5.642
0.6	0.7522	2.6	3.260	4.6	5.767
0.7	0.8776	2.7	3.385	4.7	5.892
0.8	1.003	2.8	3.510	4.8	6.018
0.9	1.128	2.9	3.636	4.9	6.143
1.0	1.254	3.0	3.761	5.0	6.269
1.1	1.379	3.1	3.887	5.1	6.394
1.2	1.504	3.2	4.011	5.2	6.519
1.3	1.630	3.3	4.137	5.3	6.645
1.4	1.755	3.4	4.262	5.4	6.770
1.5	1.881	3.5	4.388	5.5	6.895
1.6	2.006	3.6	4.513	5.6	7.021
1.7	2.131	3.7	4.639	5.7	7.146
1.8	2.257	3.8	4.764	5.8	7.272
1.9	2.382	3.9	4.890	5.9	7.397
2.0	2.507	4.0	5.015	6.0	7.522

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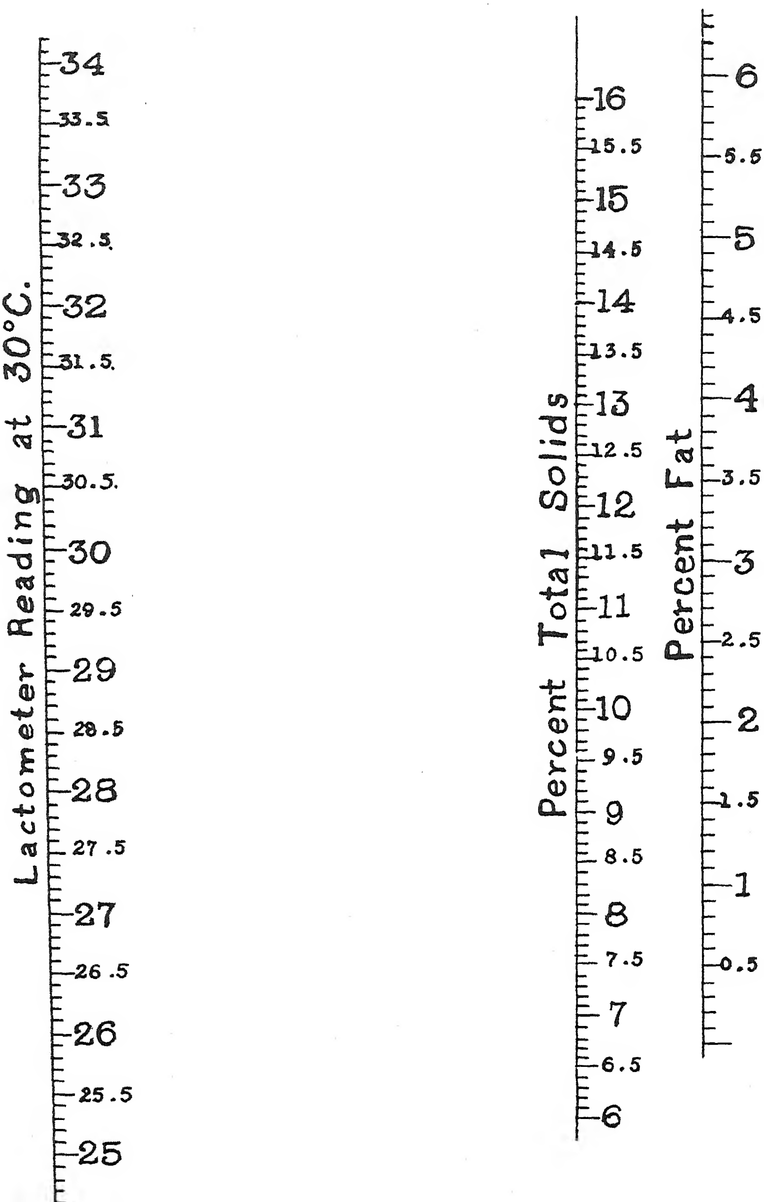

FIG. 1. Nomograph for total solids in milk according to Sharp and Hart's equation. Directions: A line or straight edge joining the lactometer reading on the left hand column with the per cent fat shown on the right hand column will pass through the center column at a point corresponding to the per cent total solids.

the total solids or solids-not-fat in milk. Inasmuch as the Sharp and Hart method has a theoretical advantage over the Babcock procedure it should be studied further. The increased length of time to prepare the sample and obtain a reading as well as the calculations needed may deter some from using the method.

Recently the writer (5) published a nomograph for finding the solids-not-fat according to the Babcock formula. Since it may facilitate at least part of the effort in using the Sharp and Hart method, the present tables and nomograph have been prepared. In table 1 factors for the fat content are given and in table 2 those for the lactometer readings, made at 30° C. A line on the nomograph joining the figures for the fat content and the lactometer reading for a given sample of milk will pass through the scale

TABLE 2

Lactometer factors for total solids in milk according to Sharp and Hart's equation

Lactometer reading at 30° C.	Factor	Lactometer reading at 30° C.	Factor
22.0	5.769	30.0	7.806
22.5	5.897	30.5	7.897
23.0	6.025	31.0	8.058
23.5	6.153	31.5	8.184
24.0	6.281	32.0	8.310
24.5	6.408	32.5	8.436
25.0	6.537	33.0	8.561
25.5	6.664	33.5	8.687
26.0	6.791	34.0	8.812
26.5	6.918	34.5	8.938
27.0	7.046	....	....
27.5	7.173	....	....
28.0	7.300	....	....
28.5	7.426	....	....
29.0	7.553	....	....
29.5	7.679	....	....

DIRECTIONS.—To find the total solids according to the equation 1.2537 fat $+ 0.2680 \frac{\text{lactometer at } 30° \text{ C.}}{\text{Sp. Gr.}}$ add the factor corresponding to the fat content in table 1 to the factor for the lactometer reading found from table 2. Example—Fat = 3.8%, Lactometer 30.5. The fat factor is 4.764, the lactometer factor is 7.897. The sum of the factors is 12.661 or the total solids content is 12.66%.

at a point corresponding to the total solids content. In making the calculations all significant figures in the Sharp and Hart equation were used. The tables will give figures to the nearest one one-hundredth per cent and the nomograph can be read to within five one-hundredths as per cent total solids.

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THE EFFECT OF PASTEURIZATION ON *ESCH. COLI* IN MILK AND ICE CREAM MIX

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For the pasteurization of ice cream mixes a temperature higher than that used for milk is generally employed. The reason for this is based partly on an improvement which results in certain physical properties of the mix and partly on the belief that *Esch. coli,* which is often considered a test organism, is more resistant to pasteurization when suspended in an ice cream mix than when contained in milk. While there is experimental evidence establishing the protective action of mix, at least for certain strains of *coli,* there are a number of questions dealing with the phenomenon which have been left unanswered, which concern particularly the nature of the protective action and the substances present in the mix which may be responsible for the increased resistance of the organism. The present study was undertaken to answer these questions, if possible, by means of quantitative data on the comparative resistance of *Esch. coli* in milk and ice cream mix.

A review of the literature reveals that but one article deals with experimental data on the comparative resistance of *Esch. coli* in the two media to the pasteurization process as ordinarily practiced. In this report, by Fabian and Coulter (1), the results of tests on forty-four strains of *coli* and aerogenes are described. Cultures of each strain were inoculated into ice cream mix and skim milk, held for two hours at 37° C. and then pasteurized. Subcultures were made into lactose broth and the success or failure of the pasteurization process was indicated by a lack of growth or growth of surviving coli in this media. At 144.5° F. no strains survived in milk. In ice cream mix the results were variable, in different experiments as few as two and as many as ten of the 44 cultures tested survived. While no data are given in the report on the number of organisms at the start of heating, it appears from the number inoculated that the strains which survived were of unusual resistance. It is likely also that the ice cream mix cultures contained greater numbers of organisms than the milk cultures. These authors also studied the individual ingredients of the ice cream mix but were unable to demonstrate a protective action which could be ascribed to any one ingredient. Mention of this point is important inasmuch as their paper has on a number of occasions been quoted as demonstrating the protective action of sugar. In general, while these experiments seem to show that an ice cream mix exerts a protective action on certain strains of the colon bacillus, they offer no suggestions as to the cause of the protective action. In addi-

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tion they do not show whether there is a general increase in the resistance of the organisms or whether the apparent increase in survival is due to some other cause such as the preservation of clumps by the high viscosity of the mix.

In addition to the studies of Fabian and Coulter, there are a number of publications dealing with the protective action of hypertonic sugar solutions. These, which will be discussed below, have for the most part been made at temperatures lower than those which are usually used for pasteurization or with stronger sugar solutions than are used in practice and therefore do not bear directly on the problem under discussion.

In our own studies the possible protective action of ice cream mix was studied in the following way. Samples of milk and commercial ice cream were heated momentarily to 100 degrees centigrade, then cooled rapidly in order to destroy any *coli* organisms already present and to reduce to a minimum the numbers of other organisms. Three ounce quantities of milk or mix in four ounce bottles were then inoculated with *coli* cultures and allowed to incubate at 37° C. for three or four hours. The *coli* culture used was a 24 hour old culture of a human strain recently isolated. At the end of the incubation period, the bottles were placed in a deep water bath regu-

TABLE 1

Survival of Esch. coli in milk and ice cream mix at 143° F.

Experiment number	No. organisms before heating	No. of survivors after				
		10 min.	20 min.	25 min.	30 min.	35 min.
A. Milk						
1	1,000,000 (2.6)	0.0	0	0		
2	" (2.7)	3.7	0	0		
3	" (0.2)	450.0	0	0		
4	" (0.7)	0.0	0	0		
5	" (2.0)	0.0	0	0		
6	" (0.7)	29.0	0	0		
7	" (1.1)	0.0	0	0		
8	" (0.9)	0.0	0	0		
Average	1,000,000 (1.4)	60.4				
B. Ice Cream Mix						
1	1,000,000* (2.6)	6400.0	1500.0	170.0	18.0	35.0
2	" (2.2)	7700.0	0.4	0.0	0.4	0.4
3	" (0.5)	120000.0	1600.0	36.0	0.0	0.0
4	" (1.8)	890.0	0.6	1.2	2.4	1.2
5	" (16)	5100.0	180.0	5.0	0.3	0.0
6	" (1.0)	2100.0	14.0	2.0	1.0	1.0
7	" (0.6)	1100.0	33.0	0.0	0.0	0.0
8	" (0.5)	44.0	2.0	0.0	0.0	0.0
Average	1,000,000	18000.0	416.0	27.0	2.8	0.2

* The data have been calculated on the basis of an initial one million organisms. The actual counts in millions are given in parentheses.

lated at 143° F. The milk attained the temperature of the bath in approximately six minutes; the bottles of mix in about seven minutes. Counting from the time at which the temperature of the materials reached 143° F., one-ml. quantities of milk or ice cream mix were withdrawn at suitable intervals, cooled by diluting in 99 ml. of sterile tap water and plated in this and higher dilutions on eosin-methylene-blue agar. After 48 hours of incubation at 37° C. the *coli* colonies were counted. The largest quantity of undiluted material which was plated out was 0.01 ml. and the results were calculated on the basis of this volume. However, the figures in the table may be read as applying to any other unit of volume. Thus, referring to Experiment 1 of the ice cream series, an initial 1,000,000 organisms would yield 6400 survivors whether the original organisms were contained in 0.01 ml. or in 1 ml.

The results of eight tests on ice cream mix with the corresponding control tests on milk are given in table 1.

The data in the table have too wide a variation as between experiments for any degree of refined mathematical analysis of the average values. They do show, however, very clearly that commercial ice cream does exert a protective action for *Esch. coli.* Inspection of the figures in the table indicates that the same percentage reduction in the number of living organisms requires about twice as long in ice cream as is required in milk. The fact that in each experiment in ice cream mix there is a progressive reduction of numbers of survivors, with time, strongly suggests that the general level of resistance of all the organisms present is raised rather than that of a few in the suspension. This fact in turn argues against the chance that the end result is due to the survival of organisms buried in clumps.

Having confirmed the fact that commercial ice cream mix protects *coli* against the action of heat, there remained the search for the individual ingredient which might be responsible for the protective effect. It was assumed that some ingredient would be responsible, although the possibility was recognized that certain conditions of colloid structure, rather than the presence of a single substance might ultimately be found to underlie the phenomenon under investigation.

Proceeding on the first assumption, experiments were run in which single ingredients of ice cream were added to milk, *coli* organisms added, and the mixture pasteurized at 143° F. The results are summarized in table 2.

It will be noted that butter, flavor, color, sugar (liquid or granulated), and gelatin were without influence on the survival of *Esch. coli* at the pasteurization temperature. On the other hand, sodium alginate and locust bean gum very clearly increased the apparent resistance of the organism to the pasteurization temperature. The degree of protection is approximately that observed in the commercial ice cream, although the effect may be enhanced by the presence of other ingredients. A possible objection to the experiments just cited lay in the fact that the materials were dissolved in

TABLE 2

Influence of individual ingredients of ice cream on the survival of Esch. coli in milk at 143° F.

Added ingredients	Experiment number	Colony count before heating	Number of survivors per initial million after exposures of				
			10 min.	20 min.	25 min.	30 min.	35 min.
Butter	1A	1,000,000 (17)	23	0	0	0	0
	1B	" (9)	11	0	0	0	0
	2A	" (19)	0	0	0	0	0
Flavor	2B	" (6)	33	0	0	0	0
	2C	" (10)	0	0	0	0	0
	3A	" (14)	7	0	0	0	0
Color	3B	" (23)	0	0	0	0	0
	3C	" (14)	0	0	0	0	0
Granulated sugar	4	" (8)	25	0	0	0	0
Liquid sugar	5	" (40)	16	0	0	0	0
Sodium alginate*	6	" (25)	2500	5.0	0.75	0.25	0
Gelatin	7	" (14)	7	0	0	0	0
Locust bean gum*	8	" (19)	4700	11.0	6.0	1.0	0

* 0.5% final concentration.

milk, without an adjustment of pH. Sodium alginate tends to make solutions alkaline which generally increases resistance, whereas our gelatin and locust bean gum reacted acidic, thus favoring the action of heat. In order to study this pH effect another series of experiments was run, adding the sodium alginate, locust bean gum and gelatin to water and to buffer at 6.7. The results are summarized in table 3.

TABLE 3

Survival of Esch. coli at 143° F. in solutions of stabilizers at different pH

Stabilizer	pH of solution	No. organisms before heating	Number of survivors per initial million organisms after exposures of				
			10 min.	20 min.	25 min.	30 min.	35 min.
Gelatin	4.8	1,000,000 (9)	0	0	0	0	0
	6.7	" (16)	6.3	0	0	0	0
Locust bean gum	4.3	" (9)	145	0.6	0	0	0
	6.7	" (19)	4800	11	6	1.1	0
Sodium alginate	8.1	" (6)	2700	50	0	3	0
	6.7	" (18)	330	1.7	0.5	0	0
Buffer control	8.0	" (19)	0	0	0	0	0
		(3)	0	0	0	0	0

The gelatin used gave a pH in water of 4.8. At this pH and at pH 6.7 the killing was approximately as rapid as in milk or in buffer alone. Locust bean gum, which gave a pH of 4.3 in water showed a somewhat greater survival at 6.7 than at 4.3. Sodium alginate, on the other hand, which unbuffered yielded a solution of 8.1, was not quite so effective at 6.7 as at this pH but still offered a considerable protection to the organisms suspended in

its solution. These results show that in commercial ice cream mix as prepared at present the substances which protect the *coli* organism against the action of the temperature of 143° F. are the stabilizers, locust bean gum and sodium alginate.

As a further test of the necessity of the stabilizers, an ice cream mix was prepared in the laboratory according to a commercial formula, with milk, condensed milk, heavy cream and granulated sugar. At 143° F. per 1,000,000 original organisms, less than 16 survived ten minutes; none survived as long as twenty minutes. The killing action in this medium therefore closely approximated that in pure milk.

DISCUSSION

Our experimental findings show the peculiar protective action of sodium alginate and locust bean gum for *Esch. coli* against the effects of heat, and also indicate that an ice cream mix without these substances is no more difficult to pasteurize than milk. The positive results of Fabian and Coulter were apparently obtained in the absence of both stabilizers which we have found responsible for protection. In seeking an explanation for this discrepancy a number of points may be considered. At 143° F. Fabian and Coulter find that only certain strains appeared to be more resistant in ice cream mix than in milk. In addition, they incubated their cultures for a period of two hours with their ice cream mix, which has been found by Fay (2) considerably to increase the resistance of *coli* to heat. The period of incubation may also have increased the differences in the counts of milk and ice cream mix suspensions so that actually more organisms per unit volume were heated in one case than in the other. Other conditions being equal, a longer time would be required to produce the same degree of sterilization for the suspension containing the larger number of organisms.

A number of workers have found that hypertonic solutions of sugar increase the resistance of *Esch. coli* and other organisms to heat. The literature has been reviewed by Fay. Of particular interest are the observations of Robertson (3) who found that a culture of *Esch. coli* was more resistant to pasteurization at 143.6° F. in 23.1 per cent sucrose than in a 1 per cent solution of this sugar. With higher concentrations of sugar progressively greater degrees of resistance were noted. At the present time the concentration of sucrose in ice cream mix rarely exceeds 15 per cent so that it can only be inferred that an increased resistance would have been demonstrated in ice cream mix. Fay, working with approximately 15 per cent sucrose found that the resistance of *coli* was increased on the average of 2.5 times by this concentration. The experiments were conducted, however, at 130° F. with an exposure of nine minutes. It cannot be assumed that the same degree of increased resistance would be effective at higher temperatures. Fay has shown that the protective action of sugar against the coagulation

of egg white falls off very rapidly with increase in temperature and it is reasonable to assume that such should be the case with organisms. In each instance the protective action is probably connected with the dehydrating and water-binding properties of strong sugar solutions—both dried organisms and dried proteins being markedly heat resistant. As the temperature of such hypertonic solutions is raised, more and more free water is produced in the mixture until at some point the unbound water occurs in such proportion that the reaction with protein goes on essentially at the rate at which it would without sugar present. Sodium alginate and locust bean gum might well work in the same manner as sugar by binding water and it would be expected that with higher temperatures the protective action of these stabilizers would become less, becoming undiscernible above some given temperature as yet undetermined. Whether or not the temperature of 155° F. is safely above this temperature is a point which will require further investigation, but from the experience of one of us (C.P.) in the practical testing of ice cream mixes, it would appear that the general absence of *coli* from pasteurized ice cream mixes, other conditions being satisfactory, indicates that this temperature is above the critical one for the stabilizers in common use at the present time. Fabian and Coulter in their experiments with various strains of *coli* found none which survived 155° F. for thirty minutes.

A point worthy of consideration is the effect of the protective substances on other organisms. While our own work was limited to *Esch. coli* the possibility is suggested that other organisms may show a similar heat resistance in the presence of certain stabilizers. Oldenbusch and co-workers (4) found a slight increase of resistance when the survival of staphylococcus, typhoid, and streptococcus was compared in milk and ice cream mix. While this difference would appear to be significant these authors concluded that it was within the limits of their experimental error. Robertson noted increased resistance for *Streptococcus thermophilus, Escherichia coli, Micrococcus aureus,* and *Sarcina lutea* in the presence of 23 or more per cent of sugar. Fay, working with sugar solutions at a lower temperature and for a shorter time than is ordinarily used in pasteurization, found that in the presence of sugar some organisms show increased resistance while others were uninfluenced. *Staphylococcus aureus* and *Aerobacter aerogenes* belonged to the former group. While further work is needed before generalizations can be made it would appear not unreasonable to assume that some organisms, including possibly some pathogens, might, with *Esch. coli,* constitute a group whose resistance to heat is increased in the presence of certain substances.

SUMMARY AND CONCLUSIONS

1. Comparative studies on milk and commercial ice cream show that the latter exerts a protective action for *Esch. coli* against the pasteurization

temperature of 143° F. The apparent resistance of the organism is approximately doubled.

2. In commercial ice cream the substances responsible for this protection are the stabilizers, locust beam gum and sodium alginate. Sugar and gelatin were found to exert no protective effect.

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EFFECT OF FREE FAT ACIDS OF MILK FAT ON CURD TENSION OF MILK. RELATION TO MILK ESTERASE, TEMPERATURE, USE OF $CaCl_2$, KIND OF FAT ACID, MILK LIPASE AND CHURNING*

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In a previous paper (1) presented jointly from this Division and the Division of Dairy Industry, University of California, preliminary evidence was given that raw skim milk will hydrolyze diglycol laurate and that this prevents the normal clotting of milk by rennet at 35° C. We are presenting in this paper the experimental evidence obtained in this laboratory in explanation of this phenomenon as well as further studies regarding various other related factors which may adversely affect the clotting properties of milk or which may overcome this tendency.

EXPERIMENTAL

General Procedures and Methods. Dispersion of esters and fat acids employed in the various studies was effected by vigorous mechanical agitation for 30 minutes, whenever possible at a temperature above the melting point of the dispersed substance. When temperature control was necessary in any experiment or part thereof, an electrically heated constant temperature water bath was employed. A glass electrode pH meter was used almost exclusively for measuring acidity. Curd tension was obtained in some cases by means of the Hill knives and spring balance and in other by the Submarine Signal Company curd tension meter.

1. *Evidence for enzymatic hydrolysis of diglycol laurate by milk.* It seems proper to refer to such an enzyme as an esterase since a simple fat acid ester is the substrate. It is probably one of the "lipases" of milk studied recently by Krukovsky and Sharp (2, 3), Herrington and Krukovsky (4, 5, 6), Mattick and Kay (7), and others. The conditions favorable for the liberation of lauric acid from diglycol laurate by raw milk and milk products resemble closely those developed by these workers for demonstrating lipolysis in raw milk. Typical experiments which collectively show that a milk enzyme decomposes diglycol laurate are summarized in table 1. Apparent variations in extent of hydrolysis are due to the work having been done at different times on different samples of milk. The increase in acidity, measured chiefly as pH, occurred at 10° C. or lower, except in experiment 8.

The data show (a) that the lauric acid ester is hydrolyzed in raw skim

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TABLE 1

Change in pH various products containing added diglycol laurate, indicating enzymatic hydrolysis of the ester

Exp. no.	Kind of product	Proportion of product to ester		Aging temp.	pH		
		Product	Ester		Immediately	1st period	2nd period
				°C.			
1	Raw skim	100	0.0	10	6.60	6.62 (36)[a]	6.60 (49)
	Raw skim	100	0.5	10	6.65	6.28 (36)	6.18 (49)
2	Raw rennet serum[b]	100	0.0	1	6.59	6.58 (18)	6.57 (42)
	Raw rennet serum	100	0.5	1	6.65	6.52 (18)	6.42 (42)
3	Raw rennet serum ultra filtrate[c]	100	0.0	1	6.59	6.60 (18)	6.58 (42)
	Raw rennet serum ultra filtrate	100	0.5	1	6.65	6.57 (18)	6.54 (42)
4	Raw acid serum ultra filtrate[d]	100	0.0	1	6.57	6.59 (15)	6.59 (40)
	Raw acid serum ultra filtrate	100	0.5	1	6.61	6.62 (15)	6.62 (40)
5	2.5% Ca phosphocaseinate sol	100	0.0	10	6.50	6.53 (36)	6.53 (49)
	2.5% Ca phosphocaseinate sol	100	0.5	10	6.65	6.65 (36)	6.63 (49)
6	2.5% Ca caseinate sol	100	0.0	1	7.13	7.13 (20)	7.13 (44)
	2.5% Ca caseinate sol	100	0.5	1	7.20	7.18 (20)	7.12 (44)
7	Egg white sol[e]	100	0.0	1	6.77	6.87 (16)	6.95 (40)
	Egg white sol	100	0.5	1	6.75	6.98 (16)	7.05 (40)
8	Raw skim[f]	2	0.5	38	7.70	7.38 (16)[g]	....
	Pasteurized skim	2	0.5	38	7.75	7.63 (16)[h]	....

[a] The numbers in parentheses indicate the number of hours the sample was held.
[b] Prepared by rennet coagulation of raw skim milk at 40° C., serum centrifuged and filtered through paper on Büchner funnel, using suction.
[c] Same as Exp. 2 except filtered by suction through a Berkfeldt filter.
[d] N/10 HCl was added to raw skim milk to pH 4.6 and the acid serum filtered through Berkfeldt filter and filtrate adjusted to pH 6.6 using N/10 NaOH.
[e] Raw egg white was diluted with 9 volumes distilled water; 20% M/1 NaCl added to disperse the globulin and the pH adjusted from 8.1 to 6.77 using 0.6% H_3PO_4 solution.
[f] In this experiment one ml. skim milk was added to 50 ml. 0.5% colloidal emulsion of diglycol laurate + one drop formalin.
[g] The titratable acidity of this sample increased 100 per cent.
[h] The titratable acidity of this sample increased 22 per cent.

milk and in rennet whey from raw milk; (b) that the hydrolysis is brought about by small amounts of raw skim milk on incubation in the presence of formaldehyde but not by pasteurized skim milk; (c) that the catalytic agent is largely, if not completely, removed from raw rennet whey and raw acid serum by a coarse ultrafilter (Berkfeldt filter); and (d) that the catalytic agent is not present in raw egg white sol, synthetic calcium caseinate sol or synthetic calcium phosphocaseinate sol.

2. *Relation of temperature, lauric acid and use of $CaCl_2$ to pH and curd tension of milk treated with diglycol laurate.* It was shown in the previous paper (1) that the conditions found by Tarassuk for demonstrating the

impaired rennet clotting properties of raw skim milk to which diglycol laurate has been added are (a) proper dispersion of the ester into the milk at room temperature, (b) aging the emulsion at low temperature, (c) warming the emulsion to clotting temperature. Having discovered that hydrolysis of the ester by milk esterase is the cause of the decrease in pH during the period of aging at low temperature, experiments were conducted to compare the clotting ability of ester treated milk with that of milk to which free lauric acid was added. Several experiments of this kind were carried out, but only typical results will be reported. In all cases sufficient lauric acid was vigorously emulsified in the milk for 30 minutes at 45° C. (this is slightly above the melting point of the acid) to lower the pH to that attained in the non-clotting, ester-treated milk after aging. The experiments also included a study of the favorable effect of adding $CaCl_2$ in the curd tension test, an effect already observed by Tarassuk (1).

In the course of these studies we found that the free lauric acid added to the milk would impair the clotting ability only after aging.[a] Milk containing freshly emulsified lauric acid exhibits normal curd tension.[a] Evidently the gradual decrease in curd tension shown by milk containing added diglycol laurate at low temperature is not wholly due to the time necessary for the liberation of lauric acid. The primary purpose of the low temperature was to prevent lactose fermentation, but it seems very probable from later observations that the same effects could be produced at temperatures perhaps as high as 20–25° C. if lactic acid development could be excluded. However, a bacterial antiseptic would have to be used which does not in itself interfere with normal clot formation by rennin. We did find that neither milk in which lauric acid was liberated from the ester nor the milk to which the acid was added would show impairment of clotting ability if the curd tension test is performed at 40° C. instead of at the normal temperature of 35° C. or if the milk is first heated momentarily to 50–55° C. before bringing it to 35° C. for the curd test.

Results bearing on several of these conclusions are shown in table 2. Experiments II, III and IV were performed simultaneously on the same lot of raw skim milk. Attention is directed to the markedly similar results obtained from the hydrolyzed lauric acid ester and the added lauric acid. Experiment IV shows that the effects of the acid are completely nullified at 35° C. if $CaCl_2$ is used in the curd test and that this is associated with a rather marked further decrease in pH. Experiment II shows that complete loss of clotting ability is attained more rapidly when the lauric acid is added directly to the milk. This would be expected.

Experiments I, II and III, table 2 show how greatly the effects of both

[a] It has subsequently been observed that some samples of milk do exhibit a substantial reduction in curd tension immediately after addition of lauric acid. The divergent results are being investigated.

TABLE 2

Comparative effects on curd tension and pH of hydrolysis of diglycol laurate and added lauric acid and relations thereto of temperature and $CaCl_2$

Sample no.	Description of sample	Curd tension[a]			pH		
		A	B	C	A	B	C
		gm.	gm.	gm.			
	Experiment I (40° C.)						
1	Raw skim + 0.5% dg.l.[c]	68	50 (24)[b]	78 (48)	6.65	6.47	6.42
2	Raw skim + 0.3% l.a.[d]	104	74	....	6.18	6.30	....
	Experiment II (35° C.)						
3	Raw skim + 0.5% dg.l.	63	17 (24)	0 (48)	6.48	6.38	6.37
4	Raw skim + 0.3% l.a.	....	4	....	....	6.32	....
	Experiment III (35° C.)						
5	Raw skim + 5% dg.l. △ 55° C.[e]	....	76 (24)	....	....	6.38	....
6	Raw skim + 0.3% l.a. △ 55° C.	....	68	....	....	6.32	....
	Experiment IV (35° C.)						
7	Raw skim + 0.5% dg.l. + $CaCl_2$	70	83 (24)	88 (48)	5.75	5.68	5.67
8	Raw skim + 0.3% l.a. + $CaCl_2$	....	103	....	....	5.60	....

[a] Determined by Hill knives at temperature indicated after number of each experiment, using one ml. concentrated rennet extract to 100 ml. milk. When $CaCl_2$ was used, as in Exp. V, 2.5 ml. Hill $CaCl_2$ sol. were first added to 100 ml. of milk. Data represent average of duplicate determinations.

[b] The numbers in parentheses indicate the number of hours the samples for that experiment were aged. Only subsamples B and C were aged at low temperatures, the data in column A being obtained on the freshly prepared emulsions.

[c] dg.l. = diglycol laurate. [d] l.a. = lauric acid.

[e] The figure after △ indicates the temperature to which the sample was heated momentarily before bringing it to 35° C. for the curd tension test.

added and liberated lauric acid are modified by the clotting temperature employed or by flash heating to 50–55° C. Since the melting point of lauric acid lies between 40° C. and 50° C.,[1] the results do not seem to be related to the physical condition of the acid. The duration of holding the samples at 40° C. was not controlled in Experiment I, which might account for the somewhat variable curd tensions obtained at the different periods. Therefore, it seemed of interest to determine the rapidity with which a lauric acid treated milk, aged in the cold, would regain its normal clotting property at 40° C. and also whether the same result would follow if the milk were held for a sufficiently long time at 35° C. A typical experiment is shown in table 3. The curd tests in this case were made at 35° C. without $CaCl_2$, using the Submarine Signal Company curd tension meter.[2] The milk was raw market skim milk. Undiluted rennet extract was added at the rate of 1 ml. per 150 ml. milk. It is evident that normal curd tension of lauric acid treated milk which has been aged in the cold may be restored within one-half hour at 40°

[1] The lauric acid used had a melting point of 44° C., which was reduced to 42.5° C. by emulsifying in water or whey.

[2] A comparison of the curd tension of a number of samples of normal milk by the Hill knives with the surface cut and body cut values given by the Submarine Signal Company curd tension meter showed that the Hill value always corresponded most closely with the surface cut value.

C. and that there is also considerable restoration of normal clotting properties at 35° C. The question whether this will ever be complete at the latter temperature is not satisfactorily answered in the experiment reported since the result with the five-hour sample is complicated by a drop in pH which undoubtedly assisted the restoration of curd tension. However, in another experiment at 35° C., not reported here in detail, in which the pH did not change, only about 75 per cent of the original curd tension had been restored at the end of six hours.

TABLE 3

Restoration of curd tension determined at 35° C. of aged, lauric-acid-treated skim milk, by conditioning at 35° C. and 40° C., respectively

Description of sample	Temp. of conditioning	Time of conditioning	Curd tension*		
			Surface	Body	pH
	°C.	hr.	gm.	gm.	
Fresh emulsion	35	0.0	50	39	6.28
Aged emulsion	35	0.0	0	0	6.30
Aged emulsion	35	0.5	3	8	6.33
Aged emulsion	35	1.0	14	22	6.30
Aged emulsion	35	2.0	18	22	6.28
Aged emulsion	35	5.0	50	37	6.18
Aged emulsion	40	0.0	3	10	6.33
Aged emulsion	40	0.5	50	43	6.33
Aged emulsion	40	1.0	53	45	6.30

* Submarine Signal Company curd tension meter values.

3. *Restoration of normal clotting by addition of $CaCl_2$.* A direct effect of lauric acid on rennin itself first had to be ruled out before the effect of $CaCl_2$ could be studied more closely. To this end the clotting property of two portions of commercial rennet extract[3] was compared after they had been treated as follows. Portion A, warmed to 40° C., was diluted with 25 per cent melted lauric acid and the mixture emulsified for 30 minutes at this temperature. The ratio of rennin to lauric acid in this emulsion is approximately that found when adding one ml. rennet extract to 100 ml. milk containing 0.3 per cent lauric acid. Portion B was treated similarly with water. Graded amounts of the two portions were then added to 100 ml. portions of raw skim milk at 35° C., and their curd tensions determined by the Hill knives without adding $CaCl_2$. The results are tabulated at top of p. 434.

The evidence is slight, if any, that the contact of the rennin with the concentrated emulsion of lauric acid affected its activity under the conditions of the experiment.

The nullifying effect of $CaCl_2$ on the action of lauric acid was first examined from the standpoint of a chemical reaction occurring between the salt and the acid. This was determined indirectly through the effects on

[3] The rennet extract used throughout this study was kindly furnished by Chr. Hansen's Laboratory, Inc., Milwaukee, Wis.

Amount rennet extract added per 100 ml.	Curd tension when using	
	Lauric acid treated rennet A	Water treated rennet B
ml.	*gm.*	*gm.*
1.3	58	67
1.0	68	72
0.7	62	62
0.4	45	55

curd tension rather than by isolating the products of such a reaction. For this purpose two portions of raw skim milk at 45° C. were subjected to a 30-minute mechanical emulsification respectively with (A) 0.3 per cent lauric acid and (B) 0.25 per cent Hill $CaCl_2$ reagent[4] which had been heated at 80° C. for five minutes with 10.7 per cent lauric acid. The latter mixture contained $CaCl_2$ and fat acid in chemical equivalent (approximately) amounts. Both portions were aged for 24 hours at 0–5° C. and their curd tension obtained at 35° C. by the Hill knives. Portion A had zero curd tension, portion B a curd tension of 73 grams. This result indicates that a product is produced by allowing $CaCl_2$ and lauric acid to react chemically which exerts no effect on the clotting of milk with rennin.

The question was next examined whether the low pH of $CaCl_2$-treated milk, to which attention was called previously in connection with Experiment IV, table 2, plays a role in nullifying the effects of lauric acid. It is difficult to devise an experiment which will give an unequivocal answer to this question. Any acid which lowers the pH of milk to the acidity found in the experiment cited will itself increase the calcium available for combining with fat acid. However, it was hoped that some light might be thrown on the question by comparing the restoration of curd tension in milk rendered non-clotting through added lauric acid (a) when the pH only was reduced and (b) when a rather large increase in calcium concentration was produced without changing the pH of the non-clotting lauric acid milk. A 1.0 N solution of HCl was used for the pH adjustment and mixtures of the Hill $CaCl_2$ reagent and calcium sucrate solution[5] were used to increase the calcium content without changing the pH. The effect of these reagents was tested on two portions of the same lot of milk, one portion of which was emulsified for 30 minutes at 45° C. with 0.3 per cent lauric acid and aged for 24 hours at 0–5° C., and the other portion, which served as

[4] Calcium chloride is usually regarded as an acid compound because of its pH reducing effect when added to milk. However, it does not seem to be generally recognized that aqueous solutions of $CaCl_2$ are slightly alkaline, showing that the salt has a greater tendency to form $Ca(OH)_2$ than HCl in water. The pH of the Hill curd tension $CaCl_2$ reagent is 7.7, which on dilution (2.5 ml. to 100) with distilled water rises to 8.1.

[5] The calcium sucrate was prepared by saturating a 33 per cent sucrose solution with $Ca(OH)_2$, and filtering off the surplus lime.

control, treated in the same way without the addition of lauric acid. Curd tensions were determined at 35° C. by the Hill knives.

The results of this experiment are given in table 4. They indicate that the increase in calcium salts obtained either by direct addition or indirectly through an increase in acidity are probably more important in overcoming the detrimental effects of lauric acid than the increase in acidity. However, the higher curd tension shown by the control milk when HCl was added than when the calcium content only was increased suggests that a pH effect is not entirely negligible.

TABLE 4

Relative effects of calcium salts and decreased pH in restoring normal clotting properties to milk containing added lauric acid

Sample no.	Reagent added per 100 ml.	Control milk		Lauric acid milk	
		Curd tension	pH	Curd tension	pH
		gm.		*gm.*	
1	None	51	6.70	8	6.32
2	2.5 ml. Hill $CaCl_2$	55	5.85	85	5.58
3	1.5 ml. 1.0 N. HCl*	77	5.88	60	5.55
4	0.5 ml. Hill $CaCl_2$ + 0.28 ml. calcium sucrate	54	6.65	79	6.30
5	1.0 ml. Hill $CaCl_2$ + 0.56 ml. calcium sucrate	37	6.69	56	6.30

* The HCl solution was added to the cold milk by capillary tube inserted into the milk which was being mechanically stirred.

4. *Effects of other fat acids of milk fat on clotting of milk by rennin.* All of the other commonly recognized fat acids of milk fat from C_4 to C_{18}, inclusive, except myristic acid, were available for study. However, ethyl myristate[6] (b.p. 137.5° C. at 2 mm.) was tested in an experiment in which the activity of the milk esterase was depended upon, as was likewise done in the case of butyl stearate and diglycol oleate, which were also tested. The oleic acid used was a sample of high purity[7] (I_2 value 88.3). The other fat acids were of commercial origin and were probably reasonably pure products, inasmuch as they showed essentially normal physical properties.

Raw skim milk of market origin was used in order to have milk of as uniform curd tension as possible since the various fat acids could not conveniently be tested in the same milk. The raw market skim milk used was found to give quite uniform curd tensions from day to day. When a free fat acid was to be tested a preliminary experiment was usually run to determine the amount of acid required to reduce the pH to 6.2–6.3 under the

6 This was supplied through the courtesy of Drs. W. H. Lauer and F. L. Greenwood of the Division of Organic Chemistry, Institute of Technology, University of Minnesota.

7 This was supplied through the courtesy of Dr. G. O. Burr of the Department of Botany, University of Minnesota.

conditions of the experiment, namely, vigorous agitation for 30 minutes at 45° C. The agitation procedure was carried out even when the acid dissolved readily in the milk, as in the case of butyric acid. When the emulsifying temperature was below the melting point of the acid a few tenths per cent of the melted acid were added slowly to the milk at 45° C. during the agitation, with the hope that there would be sufficient dispersion and ionization to bring about the desired increase in acidity. The result was not very successful in the case of palmitic and stearic acids, as shown by the data. In any case whether the fat acid increased the acidity of the skim milk or not, the curd tension at 35° C. and pH of the freshly prepared emulsion were determined (and in some cases also that of a control which had been agitated but to which no acid had been added) and the same properties determined again after 24 hours and sometimes also after 48 hours aging at low temperature. Whenever the added acid showed an effect on the curd tension at 35° C. an effort was made to determine whether the effect could be overcome by holding the milk at 35° C. or 40° C., and also the holding period necessary to bring this about. When the fat acid esters were employed, 0.3 per cent emulsions of the esters were prepared and the experiments carried out in all other respects like those involving the lauric acid ester.

TABLE 5

Lack of effect of various fat acids of milk fat on curd tension of raw skim milk

Product added	Concentration used	Curd tensions						pH		
		Fresh		24 hr.		48 hr.		Fresh	24 hr.	48 hr.
		Surface	Body	Surface	Body	Surface	Body			
	per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
Butyric acid	0.1	63	48	63	51	......	......	6.20	6.20	....
Caproic acid	0.15	71	48	72	49	......	......	6.05	6.10	....
Caprylic acid	0.1	73	46	78	55	......	......	6.30	6.32	....
Caprylic acid	0.3	93	73	74	64	......	......	5.90	6.02	....
Ethyl myristate	0.3	51	38	55	43	54	44	6.55	6.32	6.30
Control	0.0	54	38	60	39	55	40	6.55	6.45	6.43
Palmitic acid	0.3	43	33	39	34	......	......	6.55	6.58	....
Stearic acid	0.4	46	33	45	33	......	......	6.65	6.52	....
Butyl stearate	0.3	29	24	35	28	42*	34*	6.65	6.55	6.35

* After holding this sample for one hour at 40° C. it showed curd tension readings at 35° C. of 53 (S), 38 (B) and a pH of 6.30 suggesting that the butyl stearate itself impairs the clotting properties and that this effect is gradually overcome by hydrolysis of the ester, which is accelerated by holding at 40° C.

Table 5 gives the results of the various experiments in which no effect of the fat acid could be discerned. The Submarine Signal Company curd tension meter was used and both surface-cut and body-cut values recorded. Only in the case of butyl stearate was any effect noticeable and, as explained in the footnote to the table, this cannot be attributed to the stearic acid.

TABLE 6

Effect of capric acid on curd tension and restoration of normal clotting properties at 35° C. and 40° C.

Description of sample	Temp. of conditioning	Time of conditioning	Curd tension		pH
			Surface	Body	
	°C.	hr.	gm.	gm.	
Fresh emulsion	35	0.0	50	45	6.20
Aged emulsion	35	0.0	0	0	6.20
Aged emulsion	35	0.5	0	0	6.18
Aged emulsion	35	1.0	0	0	6.18
Aged emulsion	35	2.0	3	5	6.20
Aged emulsion	35	5.0	40	29	6.12
Aged emulsion	40	0.0	0	0	6.20
Aged emulsion	40	0.5	29	31	6.18
Aged emulsion	40	1.0	50	43	6.20

Table 6 shows the results obtained with capric acid. Crystalline acid from Eastman Company, melting at 31° + C., was used. The data are very similar to those reported for lauric acid in table 3, the only difference being the less rapid restoration of the clotting of the capric acid milk on temperature conditioning at 35° C. and 40° C.

TABLE 7

Comparative effects on curd tension of oleic acid and diglycol oleate. Further effects of aging and hydrolysis at low temperature and effectiveness of temperature conditioning on restoration of normal clotting properties

Product added	Concentration	Temp. of conditioning	Time of conditioning	Time of low temp. aging	Curd tension		pH
					Surface	Body	
	per cent	°C.	hr.	hr.	gm.	gm.	
None (Control)	0.0	35	0	24	49	37	6.60
Oleic acid	0.4	35	0	0	6	6	6.28
Oleic acid	0.4	35	0	24	0	0	6.22
Oleic acid	0.4	35	3	24	16	15	6.02
Oleic acid	0.4	40	1	24	11	12	6.22
None (Control)	0.0	35	0	24	46	33	6.60
Diglycol oleate	0.3	35	0	0	11	14	6.60
Diglycol oleate	0.3	35	0	24	7	12	6.55
Diglycol oleate	0.3	35	0	48	10	13	6.40
Diglycol oleate	0.3	35	3	48	31	25	6.35
Diglycol oleate	0.3	40	1	48	27	24	6.40

Table 7 shows the results obtained with oleic acid and diglycol oleate. Contrary to the results obtained with lauric and capric acids, oleic acid had an immediately impairing effect on the clotting properties of raw skim milk, and temperature conditioning at 35° C. or 40° C. prior to making the curd tension test on the aged (at low temperature) sample was not nearly so effective in restoring the normal clotting properties. Similar results followed the addition of the oleic acid ester. It is noted that aging at low temperature caused a further impairment in clotting properties, analogous

to the lauric acid and capric acid additions. The increase in acidity obtained on aging the raw skim milk containing the added oleic acid ester was considerably less than for diglycol laurate. Whether this is due to differences in solubility and ionization of the fat acids themselves, or to differences in degree of hydrolysis of the esters was not investigated.

Another peculiarity of the oleic acid milks not brought out in the table was the appearance of a gelatinous precipitate in the sample held for sometime at 35° C. The material showed evidences of undergoing syneresis. There was no odor of lactic acid fermentation. That the phenomenon has some, as yet unexplained, significance is indicated by the fact that it also occurred in another experiment when using a much less pure sample of oleic acid, the details of which are not included in this paper.

5. *Effect of lipolysis on the clotting properties of whole milk.* An experimental study of this question is reported in table 8. In this study samples of fresh raw whole milk and separator skim milk from it, and aliquots of the same whole and skim milks which had been heated to 55–56° C.[8] for 30 minutes, were shaken vigorously for two hours in glass bottles in a shaking machine at 37° C. This should have induced lipolysis in the unheated samples, according to the results reported by Krukovsky and Sharp (3). The decline in curd tension (by the Hill knives at 35° C. without $CaCl_2$) on storage at low temperature which occurred only in the raw whole milk and the decline in pH, which was accompanied by development of butyric acid odor, support the conclusion that lipolysis of milk fat is detrimental to the clotting properties. The lipolysis was evidently too mild and too little of the fat acids especially detrimental to normal clotting liberated to duplicate the experiences with free capric, lauric and oleic acids. A com-

TABLE 8

Effect of induced lipolysis of whole milk on curd tension and pH

Sample no.	Description of sample	Curd tension		pH	
		Fresh*	After 24 hrs. aging†	Fresh	24 hr.
		gm.	*gm.*		
1	Raw whole, unagitated	54	53	6.63	6.64
2	Raw whole, agitated	32	22	6.47	6.42
3	Heated whole, unagitated	45	42	6.65	6.65
4	Heated whole, agitated	45	39	6.63	6.65
5	Raw skim, unagitated	73	63	6.62	6.61
6	Raw skim, agitated	73	69	6.62	6.61
7	Heated skim, unagitated	65	60	6.62	6.62
8	Heated skim, agitated	63	57	6.61	6.62

* These were tested immediately after agitation.
† Aged at 2° C.

[8] This temperature is stated by Sharp and de Tomasi (8) and by Mattick and Kay (7) to inactivate the lipase in cow's milk.

parison of samples 1 and 3, 5 and 7, 6 and 8, shows that the heat treatment itself had considerable detrimental effect on the strength of the rennet curd.

6. *Evidence that churning of natural cream may fail to induce lipolysis which affects curd tension of buttermilk.* Two experiments were conducted which bear on the question whether the normal churning of cream in butter making increases the lipolytic activity. If this is a normal occurrence it could account for the low curd tension of raw sweet cream buttermilk. The possibility that the agitation of churning may greatly increase lipolytic action was expressed by Krukovsky and Sharp (2). The evidence presented later (3) in support of this did not show any experiments at temperatures normally employed for manufacture of butter.

The experiments of Herrington and Krukovsky (4) seem to have a more direct bearing on the question; indeed, these authors conclude that a greater increase in fat acidity which was found to follow a longer storage of milks cooled at different rates was largely due to the agitation of churning employed to recover the fat for acidity determination, but this conclusion seems to have the fallacy that the only variable was the initial storage time, all samples being subjected to the same churning procedure and no evidence being given that the longer storage made it necessary to employ a longer churning time.

Since this question may be regarded as still open and in view of the evidence already presented that lipolysis can adversely affect curd tension under certain conditions it seemed important to obtain further experimental evidence on the possibility of a lipolysis in cream, induced by churning, causing the low curd tension of natural buttermilk.

TABLE 9

Curd tension and pH of buttermilks from churned creams prepared from reconstituted milks agitated at low temperatures

Sample no.	Description of whole milk giving creams from which buttermilks originated	Curd tension		pH	
		After 3 hr.	After 24 hr.	After 3 hr.	After 24 hr.
		gm.	*gm.*		
1	Raw skim + raw cream	55	56	6.50	6.42
2	Pasteurized skim + raw cream	42	39	6.55	6.55
3	Raw skim + pasteurized cream	49	48	6.49	6.42
4	Pasteurized skim + pasteurized cream	40	39	6.58	6.57

In the first experiment, shown in table 9, a lot of fresh raw milk from the station herd was separated immediately at 37° C. and both the cream and skim milk divided into two portions. One portion of each was heated at 65° C. for 30 minutes and the four portions then recombined to give the reconstituted milks, containing 3–4 per cent fat, described in that table. All were then agitated for one hour at low temperature (4–5° C.), reseparated,

at 37° C. and the cream portions aged for 16 hours at low temperature (4–5° C.). The creams were then warmed to 10–12° C., churned in glass Dazey churns and the curd tension of the buttermilks determined at once, at 35° C., using the Hill knives without $CaCl_2$, and again after 24 hours storage at low temperature. The results give some indication that the curd tension was affected by the heat treatment but there is no evidence from comparing samples 1 and 3, and 2 and 4, that lipolysis had been induced.

In the second experiment shown in table 10, it was planned to compare the effects of a normal churning temperature with those obtained at 25° C., when lipolysis would be more likely to be induced. There was a control lot of cream in which any lipase present was presumably inactivated by holding at 55–56° C. for 30 minutes. Additional controls consisted of samples of the original whole milk and skim milk which were also carried through the experiment. Curd tensions were determined by the Hill knives at 35° C., without $CaCl_2$.

TABLE 10

Effect on curd tension and pH of churning cream at normal churning temperature and at a temperature which should induce lipolysis

Description of sample	Churning temp.	Curd tension		pH	
		Fresh	After 24 hr.*	Fresh	24 hr.
	°*C.*	*gm.*	*gm.*		
Raw whole milk control	Not churned	65	61	6.56	6.57
Raw skim milk control	Not churned	74	68	6.58	6.59
Buttermilk from raw cream	25 – 10†	31	32	6.64	6.63
Buttermilk from raw cream	12	31	25	6.60	6.61
Buttermilk from heated cream	25 – 10†	21	19	6.65	6.65
Buttermilk from heated cream	12	24	18	6.63	6.67

* Storage temperature 2° C.

† The two temperatures given are the initial and final churning temperatures employed, as explained in the text.

In the actual carrying out of this experiment it was found very difficult to obtain butter at 25° C. None being evident after one hour of churning, the creams (both raw and heated) were cooled to 5° C. and churning continued at the lower temperature for an additional hour, again without result. These creams were then stored at the low temperature for 16 hours; after which they could be churned normally at 10° C. Undoubtedly the procedure which had to be followed in this experiment was more favorable for inducing lipolysis than that originally planned. The low curd tension found for the buttermilk samples is typical of raw sweet cream buttermilk but no evidence was found through change in pH or development of fat acids odors that lipolysis had occurred. However, the buttermilks from the heated creams showed the same effects of heat treatment evident in tables 8 and 9.

DISCUSSION

Krukovsky and Sharp (3) showed that hydrolysis of simple esters by milk is differentiated from "the true lipolysis of the natural fat" by the fact that the latter usually requires an "activation" by agitation at proper temperature. This would seem to offer a method of distinguished esterase from lipase in milk. However, the more recent work of these authors (9) showing that the "activation" mechanism seems to be merely that of altering the fat globule surface layer has no doubt changed their interpretation of the difference between the hydrolysis of simple esters and natural milk fat. Nevertheless, it is somewhat puzzling that the extent of agitation employed in dispersing diglycol laurate in raw skim milk influences directly the amount of hydrolysis subsequently obtained. This was shown in a previous paper (1). It seems impossible that violent agitation of skim milk to which diglycol laurate is added actually increases the dispersion of the ester because mere shaking of the ester with water is sufficient to produce a colloidal dispersion. Certainly the problem of natural versus artificial adsorption layers cannot be involved. Furthermore, violent agitation of skim milk would be expected to increase its oxygen content; this should decrease its lipolytic activity according to the recent paper by Krukovsky and Sharp (10).

The experiments with the various fat acids of butter fat point strongly to the conclusion that capric, lauric and oleic acids exert a specific effect in interfering with normal rennet clotting. This effect was manifested by the saturated fat acids only after aging the acid containing milk at reduced temperature. For oleic acid the effect was evident without aging, although increased by aging. Also the effect could be completely reversed by warming for a proper period of time at 40° C. or above, only in the case of milks containing capric or lauric acid. Considerable reversal occurred for these cases even at the normal curd tension determining temperature (35° C.) but the conditions employed in our experiments did not permit us to decide whether complete reversal would occur for the saturated fat acid milks at this temperature. The experiments showing the restorative effects of temperature treatment on lauric acid containing milk at first suggested that the melting point of the acid was related to the effect of heat treatment but this is completely ruled out by the capric acid results, the detrimental effects of which are manifested above its melting point.

It seems likely that the phenomenon is one of adsorption, the equilibrium of which is closely related to the temperature. The more pronounced immediate effect of oleic acid and the fact that this is not readily reversed, indeed perhaps never completely so, by temperature treatment alone, is probably related to the polar double bond of the acid, in addition to polar carboxyl group. The unsaturated bond evidently produced adsorption properties having a different temperature equilibrium. The phenomena

are being more closely investigated from this point of view, and the results will be reported later.

It is admitted that the experiments concerning the counter effect of $CaCl_2$ represent a rather superficial approach to the cause of this result. Although the experiments show clearly that allowing lauric acid first to come in contact with $CaCl_2$ in chemical equivalent amounts prevents the detrimental effect of the lauric acid, thus suggesting that the counter action of $CaCl_2$ is the result of a chemical reaction between it and lauric acid; nevertheless, insufficient information is available to permit a clear picture of the processes involved. Also, as stated previously, the possibility cannot be ignored that the reduction in pH caused by the $CaCl_2$ has a minor role in the reactions.

When the effects of the lauric acid were first discovered the possibility was at once presented that certain cases of poor clotting, particularly that of raw sweet cream buttermilk, might be the result of lipolysis of milk fat. The experiment reported with whole milk not only supports the conclusion that lipolysis may adversely affect the curd tension but is in line with the theory of Krukovsky and Sharp (3, 9) that lipolysis is greatly accelerated because of the "resurfacing" of fat globules brought about by their agitation in the liquid state. However, the churning experiments do not indicate that this is a normal occurrence during the manufacture of butter. In any case only a few of the fat acids which might be liberated will affect rennet clotting. Oleic acid, being by far the most dominant of these in butterfat, unquestionably plays the major role in interfering with the formation of a normal clot.

CONCLUSIONS

1. Raw skim milk and whey contain an esterase(s) which accelerate(s) the hydrolysis of diglycol laurate, diglycol oleate, and other esters at temperatures below 10° C. The enzyme(s) can be removed by ultra filtration and inactivated by heat.

2. The fat acids liberated from diglycol laurate and diglycol oleate in milk by enzyme activity or added directly to milk will, under proper conditions, seriously interfere with and even inhibit entirely the clotting of the milk by rennet. Capric acid is the only other common fat acid in milk fat which has this effect.

3. The adverse effects of the saturated fat acids are shown only after a period of aging at reduced temperature and addition of rennet at 35° C. (or lower), and may be completely reversed by heat treatment for one-half hour or more at 40° C. Some reversal occurs during several hours heating at 35° C. The adverse effects of oleic acid occur without aging and are not overcome completely by the same heat treatment.

4. The clot-preventing effects of lauric acid are not evident at 35° C. if $CaCl_2$ is added to the milk in the curd tension test. This is possibly due largely to a chemical reaction between the salt and the acid and (or) its

compounds, but may also be due indirectly in part to the lower pH of the $CaCl_2$ treated milk.

5. When lipolysis is induced in raw whole milk by agitation at proper temperature and subsequent holding at low temperature, some interference with normal rennet clotting results. Similar lipolytic effects are not necessarily induced in raw cream by churning, although the curd tension of the buttermilk will be low. The theory of protein denaturation, involving the fat globule protein, presented in an earlier paper (1) may be invoked to explain this result.

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VITAMIN D IN MILK—A REVIEW[1]

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INTRODUCTION

The introduction of vitamin D milk was based upon nutritional facts: (a) that an antirachitic factor is necessary for proper generation, growth and maintenance, (b) that this factor is lacking in dietary products normally used, including normal cow's milk, (c) that living standards precluded availability to humans of the natural agents by which the factor might be generated, (d) that milk contained the balance of a necessary nutritional triad, vitamin D, calcium and phosphorus, and (e) milk is generally available where there is need for the additional factor.

While these facts may be evident to the student who has reviewed the subject, less evident facts need some explication. The creation of a vitamin fortified milk simultaneously brought with it merchandising problems. Among these may be cited (a) the necessity of translation and transfer of knowledge of the biological laboratory and its precise and complicated terms into acceptable form for reading matter, radio and minds of the distributor salesmen and consumer (b) by inference there was tacit admission publicly of an inadequacy of cow's milk as a "perfect" food and (c) the change in equipment and processing methods, as well as prevailing regulations required a premium charge for the new vitamin-milk product.

Variation of Vitamin D in Milk. The preformed antirachitic quality of cow's milk is affected by secretory processes, feed quality and animal management. While the quantity occurring naturally in milk is far less than demanded by human dietary requirements, the variations and sources of the factor are of practical significance.

Range in Variations. The extreme spread of a number of reports of variations in the natural vitamin D content of milk is from 3.1 to 56.0 U.S.P. units per quart, a variation of some 18 fold. An average of the minimum and maximum levels cited in these studies is 19.3 and 31.7 U.S.P. units per quart, a variation of 1.6 fold. The amounts cited include variations due to the method of preparing the sample for assay, and the interpretation of the assay. Reference to factors affecting the variations in content of vitamin D in milk are of greater interest. This was excellently reviewed by Olson and Wallis (84).

Effect of Ration. While there have been a significant number of studies on the effect of several factors on the vitamin content of milk, the methods and results of most are insufficient to demonstrate the effects only of feed

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

quality. Wallis in carefully controlled experiments (86), depleted six Holstein cows of their reserves of vitamin D by feeding a deficient ration and keeping them from exposure to sunlight. This group of six cows was divided into three groups of two each, fed daily either 20 pounds of alfalfa hay containing 10,000 I.U. of vitamin D, 20 pounds of prairie hay containing 5000 I.U. of vitamin D or a ration containing as roughage beet pulp essentially devoid of vitamin D. The potency per quart of milk from the three groups at the beginning of the experiment was 5.4 I.U. After three months the cows consuming alfalfa secreted milk containing 13.0 I.U., those consuming prairie hay secreted milk containing 8.4 I.U., while the milk from the cows consuming beet pulp contained a quantity considered immeasurable. Only a small percentage (1–2 per cent) of the vitamin D consumed was secreted in the milk. It is interesting to note that when the vitamin intake of the cows is maintained, a reasonably uniform quantity per quart of milk during the lactation period may be expected (83). Luce, using a Jersey cow showed that the vitamin D potency of the milk of a cow protected from light was improved when the ration was changed from dry fodder to freshly cut meadow grass and clover. In studies by Krauss *et al.* (53), cows fed a high protein ration consisting of alfalfa with small amounts of corn silage secreted milk containing more vitamin D than cows fed a low protein ration consisting of timothy hay with liberal amounts of corn silage. It was concluded that these results were probably due to the greater intake of vitamin D from the alfalfa than from the timothy.

Effect of Breed. The total transfer of vitamin D from the normal feeds to milk is approximately the same for different breeds as shown by Wallis (83). When individual cows of the Holstein and Jersey breeds were fed 19,056 I.U. of vitamin D in 12 pounds of alfalfa hay daily, the percentage recovery in the milk was 0.47 and 0.51 respectively. The Holstein milk, however, contained about 10 I.U. and the Jersey 30 I.U. per quart, the fat of the Jersey milk being about 50 per cent more potent than the fat of the Holstein milk. A reciprocal decrease in vitamin D potency per gram of fat occurred as the percentage fat of the milk increased with progress of the lactation period. The milk flow of the Holstein cow was sufficiently large to make the transfer of vitamin D from the feed approximately the same for both animals. Under normal and parallel conditions of management, the milk of eight Guernsey cows was shown by Bechtel and Hoppert (5) to contain greater antirachitic value per quart than the milk of fourteen Holstein cows. Little difference in the potency of the fat from the two breeds was reported in these studies. Similar conclusions were made by Kon and Henry in England (47) who reported that the vitamin D of Guernsey and Shorthorn milks could be expressed by the ratio of fat percentages.

Effect of Lactation Period. As for other important nutritional factors, the vitamin D content of colostrum is greater than that of the milk from

the post-colostrum period. Henry and Kon, in a test of the churnings from a Guernsey cow in the fourth lactation found 1.22 units per gram in the milk of the first day, 0.56 in the milk of the next two and one half days, 0.36 in the milk of the following day interval, and 0.41 in the normal milk (48). This is found equivalent to 119, 55, 35, and 40 units per quart. Van Niekirk and Bleek reported even greater concentrations in the colostrum being computed at 236, 132 and 110 units per quart for three fractions, whereas two normal milk samples were found to be 17 and 24 units, respectively (49).

When fed a uniform amount of vitamin D, the vitamin D in the milk of one Jersey and one Holstein cow was found to be reasonably uniform over the entire lactation period (83).

Influence of Sunshine. That sunshine, which provides definite antirachitic aid to humans, properly exposed, affects the antirachitic value of the milk of cows, has been adequately demonstrated. In the second of two experiments by Luce (57, 58), a Jersey cow was housed for two periods in a dark stall and two periods in the open sunshine. Improved antirachitic quality of the milk was shown when the cow was exposed to sunshine whether a vitamin-poor dry ration or a green-feed ration was employed. Chick and Roscoe (13) Dutcher and Honeywell (16) have made observations confirming the beneficial effect of sunlight. Olson (64) raised two groups of four each of heifer calves, one kept in the sunlight, the other in a dark run-shed. Similar rations were given to both groups. The milk from one each of the heifers in their first lactation was fed to separate groups of pigs. Those pigs receiving milk from the cows housed continuously with access to sunlight showed greater weight gains and freedom from rickets than those receiving milk from the dark stall cows.

Campion and co-workers (11) showed that whether eight Shorthorn cows in groups of two each were fed a winter ration or a summer ration, the effects of exposure to sunshine were so marked as to lead to the conclusion that increases in potency in milk in the summer are due entirely to the sunshine. With a winter and summer ration respectively, fed to cows indoors, the milk contained 8.2 and 5.2 I.U. and when fed to cows outdoors, the milk contained 24.0 and 16.4 I.U. per quart, respectively. A number of investigators have correlated vitamin D potency of milk or milk fat with the prevalence of sunshine. The studies of Wilkinson (94) show excellent correlation between the vitamin D content of Scottish butter and the amount of sunshine.

Effects of Artificial Irradiation of Cows. Probably because of the known antirachitic effects of sunlight acting upon certain animals, fowl, and feeds, the use of artificial sources of radiation for the application of ultra violet radiation upon lactating animals has been investigated. Uniformity in results did not occur in these experiments. When used for feeding chickens, the milk of cows irradiated 15–30 minutes daily by use of a quartz-mercury

vapor lamp was found to have increased antirachitic potency (24, 25). The milk of cows irradiated by a quartz-mercury vapor lamp was reported to prevent rickets in infants normal at birth, but inadequate for the cure of rickets in rachitic cases (8). The antirachitic value of the milk of a lactating goat was increased decidedly by the use of mercury-vapor-arc radiations, although in this case ultimately the goat showed a negative calcium balance (71).

On the other hand, an extensive experiment has shown that application of ultra violet radiation from a quartz-mercury-vapor lamp to various quarters of lactating cows for four weeks was without significant effect upon the antirachitic potency of the milk (72). The variations observed in the course of the experiments were not as great as observed when the cows were differently managed according to seasonal factors.

Because calves have been shown to be protected from rickets with radiations from a quartz-mercury-vapor lamp (65) it is probable the response to the radiations by a milking cow is affected by such factors as the rate of production and adequacy of the ration. While exposure of cows, as well as other animals, to sunlight is a recommended practice, the economical advantage of artificial radiation for cows except in unusual cases, seems questionable.

Seasonal Effects. In view of the several factors which affect the normal vitamin D potency of milk, the occurrence of seasonal differences is not surprising, and such observations have been cited in several reports (84, 72, 74). Recently, Wilkinson (94) showed a sharp summer peak in vitamin D activity of Scottish and Danish butters. The highest, lowest and mean values in I.U. per gram were, for Scotland butter, June, 0.99; March, April and December, 0.08, mean 0.32; and for Denmark, July, 0.54, December, March, and November, 0.08, mean 0.23. Wallis and Olson (84) reported values for April, 0.23, July, 0.83 and November, 0.28 I.U. per gram. Bechtel and Hoppert (5) found the greatest values in July, August, and September and the lowest usually in February and that the potency may vary as much as 900 per cent over the seasons.

Vitamin D in Feeds. Not the least of nutritional problems is the adequacy of feeds for livestock. Since the vitamin D content of milk is, in part at least, affected by the vitamin content of the feeds consumed, and in view of the other physiological demands of the lactating cow, the provision of adequate amounts of this factor in feeds is no less important than for other factors. The method of curing and handling hays, and the selection of feeds for lactating cows has been shown to be important in making provisions for vitamin D requirements. Unfortified grain concentrates (65, 41) oils (7, 60) from corn, cottonseed, linseed and peanuts, roots and succulents as turnips, beets, cabbage, carrots, parsnips and potatoes (14) are very poor providers of the vitamin D factor.

The leafy portion of good quality green colored hay was found to be six times as potent as the stems, the concentration being 10.45 and 1.72 I.U. per gram, respectively. It is evident that overcuring and shattering of hay crops should be avoided. The use of a sun-cured leaf meal as a good antirachitic has been suggested (85). The quantities of vitamin D reported in roughage vary considerably and range from 500 U.S.P. units per pound for sun-cured alfalfa hay, 150 units in prairie hay, 27 units in New Zealand hay, and 40 units in moist corn silage (84, 15, 4). Oats straw has been found a reasonably good natural source of the factor (3). There is little evidence that vitamin D as such occurs in live plant tissues (6). But the degree of insolation of hay crops is an important factor in enriching them with vitamin D. Clover, timothy, and alfalfa hay when cured in sunlight contain significantly greater antirachitic value than when cured in the dark, or when artificially dried (70, 67, 68, 3). Besides shattering, excessive weathering of clover hay exposed to dew and rain has been shown to be destructive of the antirachitic value (70).

Dairy Cattle Requirements of Vitamin D. There is increasingly available evidence of the necessity of having adequate amounts of vitamin D in the rations of dairy cattle and calves. In calves, inhibition of growth, decline in blood phosphorus and serum calcium, and manifestations of rachitic symptoms such as stiffness of the animals, swollen joints and abnormal posture of knees and pasterns and rachitic tetany follow utilization of rations low in the antirachitic factor (65). Rachitic symptoms in calves have been produced by use of experimental vitamin-deficient rations. The development of rickets in calves fed what might be considered "normal" rations has been observed in both practical and experimental operations. The use of winter and summer whole milk with a basal ration, or of milk alone may not be a preventative of rickets (59, 12, 38). It has been shown in these studies that the development of active rickets or its symptoms in calves may be prevented by the use of adequate amounts of properly cured good quality hay, exposure to sunshine, and the use of vitamin D supplements. If unsatisfactory rations and inadequate sunshine in the winter are a common regime, then the use of vitamin D supplements for calves may be considered proper. The studies of Bechdel and associates indicate that the daily requirement of vitamin D of calves is between 250–450 I.U. per hundred pounds of body weight of which a minimum supplementary requirement was estimated to be 100 to 300 units.

The significance of adequate provision of antirachitic factor for calves as they mature into lactating animals is not at once evident, but presumably is so. Olson (64) reported that two cows confined from sunshine for three lactation periods suffered a marked fall in milk production by the third lactation, although sun-cured alfalfa was fed. In comparison with groups of calves matured in a similar manner except with exposure to direct sun-

shine, no difference in blood calcium or inorganic phosphorus, or reproduction of the calves was noted. The dependency of offspring on the maternal supply of vitamin D has been dramatically shown by the experiments of Wallis (82) wherein cows on rachitic rations dropped manifestly rachitic and deformed calves. The very frequent negative calcium and phosphorus balances observed in heavily lactating cows seem not to be corrected completely by administrations of calcium and phosphorus and vitamin D. According to Rupel and co-authors, "apparently the milk secretory function is so dominating that the effect of vitamin D additions through food or light is submerged to the point where its effect is not easily measurable. The feeding of vitamin D in yeast to milking cows at the height of their lactation did not improve the mineral balances although vitamin D was absorbed and the milk enriched in vitamin D" (65). Experiments by Wallis showed that the calcium and phosphorus of blood plasma of lactating cows kept on vitamin D deficient rations declined to one-half and one-fifth, respectively, the normal plasma levels. The cows manifested typical rachitic symptoms. When fed vitamin D the mineral losses of the animals were changed to significant retentions. It is probable the extent of response to administrations of vitamin D in these experiments (65, 82) was dependent upon the various factors such as condition of the animal, volume of milk secreted, and so on. The possibility of deficiency of vitamin D in the ration as a factor of the onset of milk fever has been frequently mentioned. A seasonal cycle of greater frequency of milk fever in January, February, March, and April when solar radiation is poorest, and the effects of antirachitically poor rations fed over a period of time is believed probable since supplementing of the ration for some time before parturition has been found beneficial (32, 69).

These studies indicate that over the long period of time, inadequate supplies of vitamin D in the ration of cows, and calves is reflected in the antirachitic quality of the milk secreted.

FORMS OF VITAMIN D OCCURRING IN MILK

There are several different forms of vitamin D, and it is probable that at least three forms occur in the different types of commercial vitamin D milk available. It is also probable that at least two forms occur in milk as elaborated by the cow. These two forms are activated 7-dehydrocholesterol or vitamin D_3, and activated ergosterol, frequently called calciferol, or vitamin D_2. Two additional forms, activated 22-dihydrocalciferol, or vitamin D_4, and 7-dehydro-sitosterol or vitamin D_5 also might be present.

Cholesterol is a normal constituent of body tissue, the blood, and milk. When acted upon by ultra-violet radiation, it is transformed into vitamin D_3 (7-dehydrocholesterol). The concentration of cholesterol in milk fat is about 0.3 per cent, and in skin fat about 19 per cent (18, 1). It is probable

the activated cholesterol formed by the solar radiation acting upon epidermis of cows exposed to sunlight is transported to the mammary gland and there secreted, in part at least, in the milk. Aside from the experiments showing the effects of sunlight (and artificial radiation) on cows and the vitamin D potency of their milk, and the correlations between seasonal trends in the potency of milk and milk fat and solar radiation, there is little direct (immediate) evidence of the transfer of this sterol activated *in vivo* to the milk. Nevertheless, in view of the ubiquity of the sterol in animal tissue, the knowledge that cholesterol is provitamin D (81, 10), and that solar radiation is of definite antirachitic value to animal life, there seems ample reason for assumption that the factor is thus transported to milk.

Ergosterol, a vegetable sterol, is believed a normal constituent of plant tissue, and is found in yeasts and molds. This substance, acted upon by ultra-violet radiation of from 2300–3100 Å, is transformed in a series of intermediate steps and products into calciferol, or vitamin D_2. Because of its vegetable origin, it is presumed that the principal sterol of forages acted upon by solar radiation when sun cured is ergosterol. Because, as will be discussed, the calciferol of irradiated yeast when fed to cows is passed through the blood stream to milk, it is also presumable that this vitamin as well as the forms D_4 and D_5 of forage crops is similarly translated to the secreted milk. It is interesting to note that for the cow, the unirradiated ergosterol evidently is not transmitted from the feeds to the milk (56). It has been suggested by Bills (8) that possibly the provitamins of vitamins D_4 and D_5 occur in the phytosterols of the plant kingdom. If so, the vitamins may be present in sun cured forage and appear subsequently in the milk of cows. The constitutional form and characteristics of the various forms of vitamin D have been adequately reviewed by Bills.

INCREASE IN VITAMIN D IN MILK BY BIOLOGICAL MEANS

It has long been known that when substances rich in vitamin D are fed to lactating animals, a definite increase in the antirachitic value for the milk can be obtained. Among the substances rich in vitamin D fed to cows and which have caused an increase in the factor in the milk are cod liver oil, irradiated yeast, irradiated ergosterol, and irradiated molds. Of these the feeding of irradiated yeast has been the most frequently studied, and proven the most practical.

Yeast, of which there are many strains, contains several sterols, among which may be included zymosterol, cerevisterol, and 22-dihydroergosterol. The amount of the activatable provitamin D ergosterol in yeast is dependent upon the strain, but in some forms may approximate as much as 2–3 per cent. A suspension of yeast, removed from the spent wort, is exposed to ultra-violet radiation, and the provitamin ergosterol is converted into the active form of vitamin D_2. When fed to cows, the vitamin D_2 is absorbed

in the blood stream and transferred *via* mammary gland to the milk. The conditions under which the yeast is fed affect the rate and completeness of transfer of the factor to the milk.

When a cow is fed irradiated yeast (108,500 U.S.P. units) the absorption of vitamin D from the intestine, as measured in the blood, begins within one to two hours and reaches its maximum by the second hour (55). Calculations indicate that practically 100 per cent of the vitamin D fed appears in the blood stream. While this transfer seems efficient, the ultimate transfer of the factor to the milk is relatively inefficient and approximates some 2 to 3 per cent (36). The major portion of the vitamin D absorbed seems to be destroyed in the body, for only a portion (25 per cent) of the vitamin D ingested is eliminated by way of the intestine. It is not believed that the vitamin D (unaccounted for) is stored in unusual amounts in the tissues, since deposits of the factor have not been located, although it may be retained over a period of weeks in various organs of some species (37). The secretion of vitamin D in the milk of a cow approximately parallels the level of vitamin D in the blood. The concentration of the factor in the blood plasma therefore seems to govern the concentration of the factor in the milk (55). The higher concentration of vitamin D in the blood after ingestion of the antirachitic feed explains the greater efficiency of transfer of the factor from the feed to the milk by high producing cows. Higher producing cows secrete a greater percentage of their total production in the first half the interval between milkings than do low producing cows. When fed the same amount of vitamin D in the ration, a greater amount of vitamin D is recovered in the milk of the high producing cow. Reduced to practice, when a desired level of vitamin D is desired per unit volume of milk, relatively less irradiated yeast need be fed cows that secrete a greater volume of milk (22). It is interesting to note that while decreased feeding of irradiated yeast caused a proportionately lower content of the factor in the milk, multiple increases of yeast in the ration were not accompanied by corresponding increases of vitamin D in the milk. The cow appears able to utilize the vitamin D effectively only within a certain range. The vitamin D recovered in milk from cows fed irradiated yeast is concentrated essentially in the fat fraction of the milk. In studies where irradiated yeast was fed cows, it was concluded that the lower the total production of butterfat, the greater the concentration of the vitamin in the fat, and further, the greater the daily production of the milk by the animal the greater will be the total number of vitamin D units excreted in the milk per day, although the number of D units per unit volume of milk may be less (36). On the other hand, it was observed by Wallis (87) that the vitamin D concentration in the blood of Jersey and Holstein cows fed daily a normal ration containing 7500 I.U., was the same, as were the concentrations in the butterfat. Since the Jersey breed produced more butterfat, the total amount of vitamin

D secreted by the breed also was high. The differences evident in the two observations are probably due in part to the use of a fortified ration on the one hand, and a normal ration on the other. The feeding of moderate supplements of vitamin D in the form of irradiated yeast to cows in amounts (60,000 I.U.) adequate for a milk product of the desired antirachitic level (400 U.S.P. units) has no significant effect upon the milk or serum phosphorus, calcium or milk ash. The feeding of supplemental quantities of yeast to cows over a period of years has not been found harmful to cows and on the basis of known physiological requirements is believed helpful.

The effectiveness of different sources of vitamin D fed cows for increasing the vitamin activity of the secreted milk is by no means the same. The vitamin D in irradiated yeast (irradiated ergosterol or calciferol) has been reported from two to three times more effective than the vitamin D in the form of viosterol (irradiated ergosterol or calciferol dissolved in corn oil) (35). On the other hand, the studies by Russell *et al.* show similar efficiencies for the two products when fed at moderate levels (60,000 units per day) and slightly better efficiencies for the yeast when the two products are fed at levels of 180,000 units per day (66). By comparison the feeding of vitamin D in cod liver oil extract (Vitex) also appeared less efficacious (51, 52). The feeding of cod liver oil as such has been shown to have a depressing action on the secretion of milk fat and is poorly if at all adsorbed from the intestinal tract of the cow (28, 23, 27). Cocoa shell, an important by-product of chocolate manufacture and a feed for cattle, acquires, during the exposure to sunshine and subsequent fermentation by yeasts, considerable amounts of vitamin D, approximately one-quarter that of average cod liver oil (46, 43). The cocoa shell contains 28–35 I.U. per gram and the cocoa shell fat about 300 I.U. per gram, unusual values for a vegetable product. When fed in levels of 32,000 I.U. daily (2 pounds cocoa-shell per cow) an increase in potency from 8.36 to 19.4 I.U. per quart was noted (computed for 4 per cent milk).

INCREASE OF VITAMIN D IN MILK BY ADDITION OF CONCENTRATES

In addition to the use of metabolic processes, the vitamin D content of milk may be increased by direct addition of the agent. Several forms of vitamin D are employed for this purpose. These are of different origin or are prepared by different methods. Of the forms used commercially the vitamin D is dispersed in a conveying agent, either of a readily miscible or dispersible oil, or in a homogeneous fluid mixture of milk constituents. These products, referred to frequently as concentrates of vitamin D, are measured in computed quantities and added to the milk to be fortified prior to its pasteurization. The amount of vitamin D in the concentrates varies for different commercial products, but approximates 4000 U.S.P. units per cubic milliliter. When used at the rate of 1.0 cubic milliliter per 10

quarts of milk, an added potency of 400 U.S.P. units per quart of milk is obtained. One ml. of concentrate added to 10 quarts of milk is equivalent to 0.0105 per cent of the original volume of milk.

Three forms of vitamin D concentrate are available (93):

(a) Vitamin D prepared from extracts of fish liver oil, and dispersed in milk constituents. The vitamin from this source is believed to consist chiefly of the cholesterol or D_3 type (8).

(b) Vitamin D (calciferol) prepared by the activation of ergosterol, either by ultra-violet, or electron radiation, and dispersed in milk constituents. A commercial source of the ergosterol is yeast. This form of vitamin D is the calciferol or D_2 type. The use of ultra violet activated – 7-dehydrocholesterol or D_3 dispersed in milk constituents in the same manner as for calciferol is entirely feasible (89).

(c) Vitamin D prepared by activation of ergosterol with ultra-violet radiation as in (b) but dissolved in an oil carrier such as sesame oil plus an emulsifying agent.

The concentrates of the forms (a) and (b) dispersed in milk constituents may consist of the vitamin dispersed in evaporated milk (17), whole milk, or products of intermediate or similar composition, or of the carrying oil dispersed in a casein solution. The concentrates of these forms are readily dispersible in milk, and require no preparation for use save removal from the container. The concentrates, consisting of vitamin D in milk constituents, are generally preserved in cans by heat sterilization. The concentrates of vitamin D in oil are biologically non-perishable, but are preferably handled with use of refrigeration.

While more frequently associated with fluid whole milk, the concentrates are used in other fluid dairy products such as chocolate and buttermilk, in evaporated milk, and ice cream, and in food products such as bakery goods.

Little experimental research has been reported on the influence of the concentrate products of vitamin D on the various properties of milk. The quantities necessary for adequate fortification of the milk are so small that effects on flavor are undiscernible, and secondary reactions have not been cited. The flavor of most canned concentrate products is quite bland. They possess perhaps a slightly cooked flavor developed in the process of sterilization. The concentrates consisting of solutions in oils possess the characteristics of the oil employed. Vitamin C in milk was found uninfluenced by the addition of a vitamin D concentrate (95).

A significant number of patents are extant on carriers, and methods of dispersion of vitamin D. Few of these are feasible for application in the dairy industry.

INCREASE OF VITAMIN D IN MILK BY IRRADIATION

The process of enhancing the vitamin D content of milk has been discussed recently in a review bulletin (92).

By the process of irradiation, milk in thin films is exposed for short periods to radiation of suitable quality and intensity so as to acquire a desired degree of antirachitic potency. Commercially, potency levels of 135 and 400 U.S.P. units per quart are attained.

The increase in vitamin D is the result of the action of radiation of wave lengths from 2300 to 3100 Å on the activatable substances in milk, believed to be principally 7-dehydrocholesterol. The sources of radiation employed are either carbon or quartz-mercury-vapor arcs. The films of milk are exposed for periods of approximately one to four seconds, respectively, at flow capacities of 350 to 1,000 pounds per linear foot per hour. The thickness of the films under these conditions of operation is approximately .04 to .06 centimeter. There are a number of factors which affect the response of milk to radiation that may be summarized as follows: composition of the milk—concentration of activatable substances and opacity of the milk to radiation; characteristics of the film—thickness and velocity; characteristics of the radiation—intensity and composition; mechanical principles—film capacity, film dimensions, distance of the source of radiation, and incidence of the radiation.

The variable mechanical factors have been controlled by employment of fixed mechanical parts and automatically operating control mechanisms. Because of the investment in equipment necessary, the economical production of irradiated milk is limited to operations capable of handling from 2,500 to 15,000 pounds or greater per hour.

The radiation, properly applied, is known to have no significant effect upon the other vitamin factors in milk. Improperly or unduly applied, the radiation may cause the development of the "sunshine" flavor, which develops also by action of solar radiation acting upon the proteins of milk. Either fluid milk or evaporated milk is processed by the irradiation method. While the antirachitic potency of dried milk may be increased by irradiation, the process generally employed consists of irradiation of fluid milk prior to its dehydration (79).

Since the publication of the review on milk irradiation (92), several developments have been announced. Of primary interest is the trend toward employment of sources of radiation having greater emission of energy in the effective ultra violet spectrum. Quartz mercury vapor arcs have been developed to consume greater inputs of energy, with consequent greater output of ultra violet radiation. Carbon electrodes too have been developed to handle greater inputs of power with greater emission of ultra violet radiation. These developments permit attainment of two objectives; greater flow capacities of milk for given operating units, and/or increased antirachitic potency in the milk. Whereas the quartz mercury-vapor arcs formerly used operated with wattage inputs of 1250, the more recent types may operate with inputs of 2700 watts. The recently introduced W carbon

electrode is reported to have approximately 50 per cent greater ultra violet emission than does the U type carbon, and may be operated at greater power inputs.

The extent of activation of milk in the various depth planes of vertical flowing films having different capacities and exposed to normally used sources of radiation has recently been re-examined (2, 63). The techniques employed in the most recent of these studies consisted in activation of milk, and of ergosterol, and the decomposition of potassium persulfate by radiation transmitted through the flowing films, and the microtome fractionation into two or three parts, of irradiated flowing films having different volume capacities. Surface halves of average films possess, generally, but slightly better than half the potency acquired by the total film. The results of application of radiation to quiescent films following intervals of turbulence appear not to differ significantly from the results obtained when films were irradiated while either continually quiescent or turbulent (94).

In a series of experiments, the source and character of the flavor in milk exposed either to solar or ultra violet radiation has been elucidated. The flavor of milk exposed sufficiently to radiation transmitted through glass, or exposed to ultra violet radiation for a sufficient period is known to originate with the protein fraction, principally the casein and albumin, of milk. The exposure of relatively pure preparations of the amino acids to radiation has led to the conclusion that cystine, tryptophane, methionine and histidine are probably the important sources of the typical "sunshine" or "activated" flavor (90, 91, 19, 20). A procedure for isolation of the fraction of milk responsible for activated flavor has been developed (21). Milk exposed to radiation for prolonged periods undergoes an increase in sulfhydryls, the concentration of which may be determined in the distillates by a colorimetric procedure.

STABILITY OF VITAMIN D IN DAIRY PRODUCTS

The vitamin D of natural or fortified food products is quite stable. The vitamin D of milk is stable to the ordinary thermal applications of pasteurization (88, 79) at 62.8° C. for 30 minutes or sterilization at 115.6° C. for 15 minutes (88), as employed for evaporated milks. A major portion of the vitamin D concentrates used for the fortification of market milk are canned and sterilized products. Vitamin D_2 (calciferol) is unstable at greater temperatures such as 232.2 to 260° C., moist state, for periods of five minutes. The naturally occurring vitamin D, and induced vitamin D, are stable during long periods of storage in products such as butter, and evaporated milk (88). The vitamin D induced in milk at experimentally high levels by ultra violet radiation (several fold more than the level attained by commercial processes) has been reported as unstable even for relatively short periods of storage (62). Vitamin D in dry yeast, stored at room temperature in a

stoppered jar, which was opened and the contents mixed at intervals, was found stable for over a period of six years (78). The vitamin D in eleven evaporated milk samples stored at normal stock room temperatures (40–110° F.) for three years was found stable in all samples after one year, and in all but a few after two and three years storage (54).

MEASUREMENT OF VITAMIN D IN MILK AND PUBLIC HEALTH CONTROL OF THE PROCESSES

Up to the present time, no reliable and accepted rapid technique has been developed for the estimation of vitamin D in milk. The official method (76) consists of the use of animal bioassay methods, requiring some three weeks for the preparation of animals, and a period of 10 days' feeding of the substances being examined. Because of the expense of conducting such tests, and the necessity for employing trained personnel, only a limited number of laboratories provide bioassay service for vitamin D measurement of foods for public service. For example, the only city-maintained vitamin D testing laboratory is that of New York City, and the only officially designated state laboratories for the testing of vitamin D in foods are those of Connecticut and Virginia. Elsewhere, the service is provided for food regulatory divisions of city health departments by arrangement with laboratories, such as at Michigan State College, Ohio Agricultural Experiment Station, Rutgers, and at private laboratories having interest in the various vitamin D processes or products.

Because of the expense of conducting a bioassay for vitamin D in a food product, the tests made upon a product of a given plant are infrequent, and may vary from periods of monthly, to quarterly, or semi-annually, depending upon the requirements of the management, or of the health regulations. Under these circumstances, regulatory officials rely principally upon the significance of clauses pertaining to labeling, unannounced sampling, and other sampling techniques. When the vitamin D milk is produced by the "metabolism method" by the feeding of irradiated yeast to cows, the yeast purchases and utilization records offer a means of checking the probable potency of the milk. Manufacturers of the yeast also provide to officials certifications of the potency of the yeast being fed to the cows. In a manner similar, producers of concentrate products of vitamin D provide certifications of the potency of the concentrates employed by milk distributors. In addition to requiring records of the use of vitamin D concentrates in milk, and the milk produced, in at least one city the addition of the vitamin D concentrate to the milk is performed by a designated agent of the health department. When the process of irradiation is employed, a recording chart indicating both uniformity of power input and milk flow are available for purposes of record.

LABELLING REGULATIONS

In the food regulations of some states, the legal definition of milk is so rigid as to prohibit the use alone of the term "milk" with any product such as milk to which has been added an adequate amount of vitamin D. Further, some regulations, by their verbal structure, prohibit the addition of anything foreign to milk, and others, properly interpreted, prohibit the addition of anything to milk. Many state regulations defining the term milk, and qualifying its use, were promulgated at a time when practices of watering, skimming and chemically preserving were more common than at present. Many regulatory officials, therefore, have not chosen to interpret the regulatory definition of the term "milk" to exclude the addition of a nutritional adjunct, since the fortification of milk with this nutritional factor (D) is in the interests of, rather than contrary to, the status of public health.

This problem has been satisfactorily handled in most of the regulatory districts by permitting the labelling of the product as "Vitamin D Milk," or "Vitamin D Added," to distinguish it from milk not containing added vitamin D. Additional requirements of labelling in some regulations is citation of the amount of vitamin D added, and the source of, or process employed in, augmenting the vitamin D in the milk.

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RULES FOR THE HERD IMPROVEMENT REGISTRY TEST*

RECOMMENDED BY THE

AMERICAN DAIRY SCIENCE ASSOCIATION

The following uniform rules have been adopted by the American Dairy Science Association for the conduct of Herd Improvement Registry Tests throughout the United States:

1. RULES MUST BE FOLLOWED.—The supervisor is not at liberty to decide as to which stipulations contained herein are essential and which are not, but is required to observe these directions in all details.

2. RELATIONSHIP OF SUPERVISORS.—The supervisor shall bear in mind at all times that he is the representative of the State College or Experiment Station and is not working for nor taking directions from the breeder, and his duty is to see that the test is honestly made and accurately reported.

3. ENTERING HERDS ON TEST.—Owners of registered cattle of any dairy breed may make application at any time to the national breed association concerned to enter their herds on test using the forms furnished by the breed association. The application should be made at least 2 weeks in advance of starting so that the breed association will have time to arrange for test supervision to apply to the first month of the herd test.

The herd test may be started at the first of any calendar month.

4. IDENTIFICATION OF COWS ON TEST.—The supervisor shall study the registration papers or official diagrams of each cow carefully and be sure that each cow he is testing is the animal for which the registration papers were issued. Because of the difficulty of identification, each cow not having a distinctive marking must bear a tattoo mark in the ear. This mark must be recorded on the official report or the test is worthless. Unidentified cows are not allowed to be placed on test, but whenever the registration papers are not at hand, a sketch must be made of the color markings and a statement made on the report as to why it was necessary to enclose the sketch. This is also necessary when the color markings do not correspond with the registration papers.

5. CONDUCT OF TEST.—Twelve consecutive official test periods of 24 hours, approximately one month apart, without preliminary milkings, shall be required during the testing year, retests and surprise tests not to be included. Credits for milk shall begin with the fourth day after calving but no butterfat test shall be made before the seventh day. The day on which the calf is dropped is counted as the first day.

6. SUPERVISION AND INSPECTION.—The conduct of the Herd Improvement Registry Test shall be in charge of the Superintendents of Official Testing in the several states. In the appointment of all supervisors and inspectors,

* Includes all revisions to December 31, 1940.

the rules for the official test covering such procedure shall apply, and all supervisors shall be appointed and employed in the usual manner.

7. COWS TO BE TESTED.—All registered cows in a herd that have ever come into milk shall be tested and remain on test as long as they are in the herd except as specified in Rule 8.

8. COWS TO BE OMITTED FROM TEST.—The following exceptions from testing may be made:

a. Cows 12 years old or over with A.R., R.O.M., R.O.P., or H.I.R. records.

b. Cows used as nurse cows throughout the lactation period having one or more A.R., R.O.M., R.O.P., or H.I.R. record meeting A.R., R.O.M., or R.O.P. requirements.

c. Cows whose registration certificates are surrendered for cancellation before the 11th month of the Herd Test Year.

9. REQUIREMENTS OF MILK WEIGHTS.—The requirement of daily milk weights is optional.

10. NUMBER OF COWS MILKED AT ONE TIME.—Not more than two cows may be milked at one time, except where a combine milker is used and then not more than four units of one section may be operated by one attendant only.

11. TIMES DAILY COWS MAY BE MILKED.—Not more than three milkings per day may be permitted.

12. NUMBER OF COWS WHICH MAY BE SUPERVISED PER DAY.—Thirty cows, or if individual samples are taken, a maximum of 60 milkings may be supervised by the tester.

13. SAMPLES TO BE TESTED.—Composite samples but not in duplicate. Individual samples may be permitted. The preliminary milking where required shall be weighed, sampled, and tested for butterfat in the usual manner. The preliminary milking shall be considered as one of the regular milkings supervised daily.

14. RETESTS. (OBLIGATORY REQUIREMENTS.)—Automatic retests are to be required when 60 days after freshening cows exceed the following daily butterfat production:

	Lbs. fat in 1 day
Mature cows	3.0
Senior 4 years old	2.9
Junior 4 years old	2.8
Senior 3 years old	2.6
Junior 3 years old	2.4
Senior 2 years old	2.2
Junior 2 years old	2.0

All retests are to be made at the owner's expense.

15. SURPRISE TESTS.—One or more surprise tests with a preliminary milking may be made by a different supervisor during the testing year. Surprise tests shall be ordered at the discretion of the breed association superin-

tendent of advanced registry and it shall be left to his discretion as to whether the surprise test be in addition to or supplant the regular test.

The first surprise test is to be at the owner's expense, others at the breed association's expense.

16. OWNER'S REQUESTED RETESTS.—The herd owner may request a retest by notifying the state superintendent of official testing within 72 hours of the close of the original test. All cows in the herd must be included in the test and at the expense of the owner.

17. REPORT BLANKS.—The American Dairy Science Association Herd Test Report Blanks are to be used to report the tests.

18. YEARLY HERD AVERAGE.—In calculating yearly herd averages the production for the herd test year of all registered cows that have ever freshened except for such cows as are omitted from test under item 8, shall be included in the herd average.

The "cow year" method of figuring herd averages shall be employed.

19. COWS WHICH ABORT WHILE ON TEST.—If a cow on Herd Improvement Registry Test aborts while in milk, and the gestation is 152 days or longer, her current record shall end and a new lactation shall begin, but if the abortion shall occur at less than 152 days the test is to be continued to complete the 305 day or 365 day test period.

20. DISHONEST OR FRAUDULENT PRACTICE.—

a. If the state superintendent of testing is satisfied that fraudulent or dishonest practices have been used in making of herd test records of registered cows, he shall report same to the breed association which may reject or cancel such records.

b. The feeding or injection of thyroid extract or thyroxine to cows on Herd Improvement Registry Test is prohibited and evidence of its use shall be cause for cancellation of such records.

21. HEALTH OF SUPERVISORS.—Supervisors conducting inspection on herds entered on Herd Improvement Registry Test shall be required to possess health certificates conforming to the same rules regarding health as required of milkers and other employees in dairies producing milk under regulations of the Standard Milk Ordinance, United States Public Health Service.

22. MATTERS NOT COVERED BY RULES.—Details of supervision which these rules do not specifically cover shall be administered by the state superintendent of official testing.

It is recommended that the Uniform Herd Test Rules of the American Dairy Science Association be used as a basis of enforcement where controversies arise.

23. REVISION OF RULES.—Any revision of the rules shall be made by a joint committee of the breed associations and the American Dairy Science Association. The chairman of the Breeds' Relations Committee shall be ex-officio chairman.

RULES FOR THE SUPERVISION OF OFFICIAL TESTS*

RECOMMENDED BY THE

AMERICAN DAIRY SCIENCE ASSOCIATION

The following uniform rules have been adopted by the American Dairy Science Association for the conduct of Official Tests throughout the United States:

1. RULES MUST BE FOLLOWED.—The supervisor is not at liberty to decide as to which stipulations contained herein are essential and which are not, but is required to observe these directions in all details.

2. RELATIONSHIP OF SUPERVISORS.—The supervisor shall bear in mind at all times that he is the representative of the State College or Experiment Station and the Breed Association and is not working for nor taking directions from the breeder, and his duty is to see that the test is honestly made and accurately reported.

3. IDENTIFICATION OF ANIMAL.—The supervisor shall study the registration papers or official diagrams of each cow carefully and be sure that the cow he is testing is the animal to which the registration papers belong. Because of the difficulty of identification, each cow not having a distinctive marking must bear a tattoo mark in the ear. This mark must be recorded on the official report or the test is worthless. Unidentified cows are allowed to be placed on test, but whenever the registration papers are not at hand, a sketch must be made of the color markings and a statement made on the report as to why it was necessary to enclose the sketch. This is also necessary when the color markings do not correspond with the registration papers.

4. POSSESSOR CONSIDERED OWNER.—For all purposes of official testing work, the possessor is considered the owner and is required to treat the animal as such, presenting all necessary data, the same as for his own.

5. NUMBER OF MILKINGS TO BE SUPERVISED AT ONE TIME.—No supervisor is allowed to supervise more than thirty-six (36) milkings per day, regardless of the method of milking—machine or hand.

6. PRELIMINARY MILKINGS.—All tests require a preliminary milking. In taking a preliminary milking the supervisor shall make certain the cow is milked dry. The exact time that this milking is made should be recorded in the barn book. The last milking of the test shall be made neither earlier nor later than the time of the preliminary milking. All preliminary milkings shall be weighed, tested, and reported and shall be considered as one of the regular milking periods with respect to the number of milkings supervised daily.

* With revisions made and adopted by American Dairy Science Association to Dec. 31, 1940. Revisions are indicated by **.

7. RULES FOR CONDUCTING THE TEST.**—Before each milking period, the supervisor shall observe that the milk pail is clean and dry and shall carry the milk pail at all times during the milking period, allowing the pail out of his hands only during the actual milking process.

Where the cows are milked by hand, only one cow may be milked at a time if in a box stall or individual quarters. Two, however, may be milked at the same time if milked by machine or if standing side by side in the stanchion line.

The supervisor must in every case be in a position to observe the milker during the entire milking process.

Milking Machines.—In case a single unit milking machine, tended by one operator, is used, two cows may be milked at a time, but no second man as stripper is allowed. The operator may strip a third cow while two cows are being milked by machine providing the cow being stripped by hand and the two cows being milked by machine are in close proximity. Double units, if used, must be operated as singles. The supervisor must keep the milking machines under as close watch as he would the person doing the milking. The supervisor shall see the tubes of the machine rinsed, and the machines put together previous to milking to make sure that they contain nothing which will affect the accuracy of the sample. The machines are to be run idle for a few minutes before being attached to the cows. The supervisor shall keep the machines under constant watch while being changed from one cow to another. He must be in sight of the machines while weighing and sampling.

Combine Milkers.—Where a combine milker is used samples may be drawn directly from the milk holder of the machine. Samples for testing may be drawn only after the milk in the container has been thoroughly agitated by releasing the vacuum in the container by admitting air through the petcock in the bottom of the container. The attendant must operate the machine so as not to release the vacuum when the milker is removed from the cow. Not more than four units of one section may be supervised at one time and but one attendant is permitted to these units. Scales on each milking unit must be found accurate by the supervisor previous to their use, and he must note that the weighing indicator points to zero before the unit is attached to each cow.

No Conversation During Milking.—The supervisor shall refrain from unnecessary conversation during the time the cow is being milked.

Right of Search.—The supervisor has the right to search the milker at any time. Refusal on the part of the milker will be construed as evidence of intent to make a fraudulent test.

Weighing Milk.—The supervisor shall weigh the empty pail before each milking. Immediately after each milking is completed he shall take charge of the pail and weigh the same on scales provided by the College and enter

the exact weight of the milk at once in his barn book, and see to it that the milker records the same weight on the owner's barn milk sheet. Any inaccuracy in the owner's scale shall be reported to the superintendent in the state concerned and to the breed association.

Sampling Milk.—The supervisor shall take a sample of the milk, being careful that the milk is thoroughly mixed. Such samples shall be kept under lock and key or in the supervisor's sight until the samples have cooled to ordinary room temperature, which is 60 to 70 degrees F. When the last samples at night are kept until morning, unless thoroughly cooled in warm weather, preservative should be added to each sample. These cooled samples shall be warmed to 70 degrees F. before samples are taken for testing.

Applying Babcock Test.—Fat determinations may be made singly. All fat determinations shall be accurately made, giving clear fat columns, or repeated until clear, well defined fat columns are secured. In case samples are tested in duplicate, or where more than one clear test is obtained on a sample of milk the average of all such tests made on that sample must be used in computing the amount of fat. If duplicate determinations vary more than .2 of one per cent the test must be repeated. Readings of the tests shall be made at a temperature of 130–140 degrees F. This temperature is to be obtained by placing the bottles in a hot water bath for 5 minutes. The samples taken at any one milking shall not be thrown away until satisfactory tests of the milking are obtained.

Testing of Acid for Adulterants.—The supervisor shall run a blank test on all sulphuric acid, consisting of 17.6 cubic centimeters of acid and sufficient water to fill the bottle.

8. LOST MILK AND LOST SAMPLES.—No substitution of lost milkings or lost samples is allowed. Any missing data due to loss of milk weights of test samples are to be left blank on the report.

9. PRELIMINARY REPORTS.—The supervisor shall report to the Superintendent of Official Testing any obvious violations of the rules of the College or the breed associations on the part of the owner or attendants, and shall report immediately any cows that meet retest requirements and note upon his report form any sickness of a cow or other conditions likely to affect the reliability of a test.

A preliminary report card shall be mailed by the supervisor from the farm to the breed association immediately after the test. Any cow not in normal condition should be reported on this card.

10. REPORT COWS WHICH ABORT WHILE ON TEST.**—If a cow on official test aborts while in milk, and the gestation is 152 days or longer, her current record shall end and a new lactation shall begin, but if the abortion shall occur at less than 152 days the test is to be continued to complete the 305 day or 365 day test period.

11. USE OF THYROXINE OR THYROID EXTRACTS.**—The feeding or injection of thyroid extract or thyroxine to cows on official test is prohibited and evidence of its use shall be cause for cancellation of such records.

12. REPORTS.—The supervisor shall make out his report so as to give all of the information asked for and then sign and mail them promptly to his appointing officer for checking and endorsement.

13. TEST PERIODS EXCEEDING TWO DAYS.—Where a test is conducted longer than the regular two days, or where more than one test is made in a month, all details of each test from beginning to end shall be reported.

14. PAYMENT OF SUPERVISORS.—Under no circumstances shall any payment, gift, or gratuity to the supervisor be made by, or permitted from the owner of the cow or anyone interested in her, and any violation of this rule will invalidate the test.

15. SUPERVISORS TO SUPERVISE TEST.—The supervisor appointed to conduct a test is the direct representative of the Superintendent of Official Testing and should be respected accordingly. His duties are to supervise tests only.

16. SWORN STATEMENTS.—Each supervisor is required to fill out a sworn statement covering all his work, which eliminates the necessity of his affirming test reports.

17. HEALTH OF SUPERVISORS.**—Supervisors conducting inspections on herds entered on official test shall be required to possess health certificates conforming to the same rules regarding health as required of milkers and other employees in dairies producing milk under regulation of the Standard Milk Ordinance, United States Public Health Service.

18. RESPONSIBILITY FOR ENFORCEMENT OF RULES.—Owners and persons in their employ, are held equally responsible with the supervisor for the enforcement of the foregoing rules. Test reports will not be accepted unless all of the above rules have been observed by all parties concerned.

American Dairy Science Association Announcements

THIRTY-SIXTH ANNUAL MEETING

UNIVERSITY OF VERMONT

Burlington, Vermont, June 23–26

Come to Vermont, the Green Mountain State, which is this year holding its Sesquicentennial celebration. Come by motor, rail or air, but be sure to come. Bring the family. Special entertainment is planned for the ladies and children. Everyone will enjoy the Green Mountains. Burlington is beautifully located on historic Lake Champlain and the University commands a wide expanse of unexcelled views of mountain, lake and valley. To the west across the lake are the Adirondacks, a perfect background for gorgeous sunsets. Many will enjoy boatrides, swimming, golf and tennis.

A reservation form and information relative to housing facilities have been sent to each member. Anyone who has not received this material or who desires same should make request to J. M. Frayer, chairman of registration committee, 489 Main Street, Burlington, Vermont.

It is our desire to make this visit to Burlington and Vermont both pleasant and profitable. The prompt filling out and return of the reservation form will greatly assist us in doing the things we would like to do for your comfort and entertainment. If you have not already attended to this matter, do it today, please.

Choice of several kinds of accommodations is available to those who make advance reservations. There are hotels, college dormitories, private homes, tourist cabins, etc.

As previously announced, all those who will attend this 36th Annual Meeting are cordially invited to visit colleges, experiment stations and other points of interest in states and provinces surrounding Vermont.

Desiring to be of service especially to those who will come by automobile, Ontario, Quebec, New Hampshire, Massachusetts, Connecticut, New Jersey and New York have all joined Vermont in preparing brief lists with locations of outstanding breeding establishments, dairy plants, and points of historic or scenic interest. These lists are now being arranged and prepared for distribution to all who signify by advance registration that they are coming.

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PROGRAM

THIRTY-SIXTH ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF VERMONT
BURLINGTON, VERMONT

JUNE 23–26, 1941

PROGRAM COMMITTEE

EXTENSION
- GLEN W. VERGERONT, Wisconsin (*Chairman*)
- J. F. KENDRICK, Bureau of Dairy Industry
- E. H. LOVELAND, Vermont

PRODUCTION
- W. E. PETERSEN, Minnesota (*Chairman*)
- K. S. MORROW, New Hampshire
- A. H. KUHLMAN, Oklahoma

MANUFACTURING
- W. V. PRICE, Wisconsin (*Chairman*)
- E. H. PARFITT, Evaporated Milk Association
- PAUL F. SHARP, New York

GENERAL
- E. S. GUTHRIE, New York (*Chairman*)
- GLEN W. VERGERONT, Wisconsin
- W. V. PRICE, Wisconsin
- W. E. PETERSEN, Minnesota

SCHEDULE OF PROGRAM

Time	General	Extension Section	Production Section	Manufacturing Section
Mon., June 23				
9:00– 3:00	Committees	Committees	Committees	Committees
9:00 ——	Directors			
2:00– 4:00				Judging
3:00– 5:00			Tour—barns and herds	
Evening 7:30	Get-together			
Tues., June 24				
9:30–11:00	Opening session			
11:00–12:00	Committees	Committees	Committees	Committees
12:00– 1:30		Lunch	Lunch	Lunch
1:30– 3:30		Papers	Papers—Div. A B	Symposium—Properties and utilization of cream
3:30– 4:30		Business	Business	Business
Evening 8:00	Reception			
Wed., June 25				
9:00–11:00		Symposium—Mastitis		Papers—Div. A B
11:00–12:00	Committees			Committees
12:00– 1:30	Picture 12:00 Barbecue 12:15			
1:30– 3:30		Papers and Panel	Papers—Div. A B	Symposium—Milk pasteurization
3:30– 4:30			Business	Business
Evening 6:00	Boat ride Buffet supper			
Thur., June 26				
9:00–11:00		Symposium and Papers	Symposium	Papers—Div. A B
11:00–12:00			Business	Business
12:00– 1:30		Lunch	Lunch	Lunch
1:30– 3:30		Papers Reports	Symposium—Curricula	
3:30– 5:00	Business			
Evening 7:00	Banquet			

REGISTRATION

Room 4-South, Old College Building

Sunday	2:00 p.m.– 9:00 p.m.
Monday	8:00 a.m.– 9:00 p.m.
Tuesday	8:00 a.m.– 7:00 p.m.
Wednesday	8:00 a.m.– 5:00 p.m.
Thursday	8:00 a.m.–12:00 m.

TENTATIVE PROGRAM FOR WOMEN

MONDAY,	JUNE 23— 2:00 P.M.	Demonstration of the Manufacture of Table Silverware — Fleming Museum.
	7:30 P.M.	Social Get-Together and Complimentary Sugar-on-Snow for Everybody —Gymnasium.
TUESDAY,	JUNE 24—11:00 A.M.	Start from Morrill Hall for Complimentary Luncheon at Mt. Mansfield Hotel on the top of Vermont.
	8:00 P.M.	Reception for Everybody—Southwick Memorial.
WEDNESDAY,	JUNE 25—10:30 A.M.	Art Exhibit Depicting Early Vermont History—Fleming Museum.
	11:45 A.M.	Group Picture—Billings Library.
	12:15 P.M.	Complimentary Barbecue Luncheon for Everybody—Gymnasium.
	2:00 P.M.	Bridge or Visiting and Tea—Southwick Memorial.
	6:00 P.M.	Complimentary Boat Ride for Everybody on Lake Champlain—Steamer Ticonderoga, Foot of King Street. Complimentary Buffet Supper on the Boat.
THURSDAY,	JUNE 26—12:30 P.M.	Start from Morrill Hall for Complimentary Luncheon at Twist O'Hill.
	7:00 P.M.	Association Banquet—Gymnasium.

Guests are particularly invited to attend the opening session of the General Program. They also will be welcome at any of the Section Programs.

FOR THE CHILDREN

Tours, Picnics, Swimming and Other Special Entertainment are being planned for Children.

GENERAL PROGRAM

Monday, June 23

7:30 P.M. Social Get-Together and Complimentary Sugar-on-Snow—Gymnasium.

Tuesday, June 24

9:30–11:00 A.M. Opening Session—Gymnasium.

Call to Order—H. B. Ellenberger, Head, Department of Animal and Dairy Husbandry, University of Vermont.

Address of Welcome—William H. Wills, Governor of Vermont and a trustee of the University of Vermont.

Response and Address—H. W. Cave, President, American Dairy Science Association.

Dairy Production Milestones—W. E. Krauss, Associate in Dairy Nutrition, Ohio Agricultural Experiment Station.

8:00 P.M. Reception—Southwick Memorial.

Wednesday, June 25

12:00 M. Group Picture—Billings Library.

12:15 P.M. Complimentary Barbecue—Gymnasium Annex.

6:00 P.M. Complimentary Boatride on Lake Champlain and Buffet Supper—Dock, Foot of King Street.

Thursday, June 26

3:30–5:00 P.M. Business Session—Auditorium, Fleming Museum.

7:00 P.M. Annual Association Banquet—Presentation of Borden Awards—Gymnasium.

SECTIONAL PROGRAMS

EXTENSION SECTION

June 23–26

Exhibits—Display of Extension Teaching Ideas
Room 1, Morrill Hall

Monday, June 23

3:00–5:00 P.M. Tour Barns and Herds—Start from Morrill Hall

Tuesday, June 24

1:30–3:30 P.M. Room 2, Morrill Hall

E. C. SCHEIDENHELM, *Chairman*

E1—Supervisory standards for state men in charge of D.H.I.A. work. R. G. Connelly and R. W. Dickson, Virginia Polytechnic Institute. 487.*

* Numbers at the end of the title refers to the page number on which the abstract appears.

E2—Revision of D.H.I.A. forms. Jos. B. Parker, Bureau of Dairy Industry, United States Department of Agriculture.

E3—Rules and regulations used in D.H.I.A. work. C. R. Gearhart, Pennsylvania State College.

E4—Survey of modified testing. E. H. Loveland, University of Vermont. 488.

E5—Methods of reporting kinds and quality of feed in D.H.I.A. work. A. J. Cramer, University of Wisconsin. 489.*

E6—New D.H.I.A. conversion factors by breeds. J. F. Kendrick, Bureau of Dairy Industry, United States Department of Agriculture. 490.

E7—Uniform annual reports for artificial breeding associations, including standardized methods of evaluating conception rates. Ralph A. Corbett, University of Maine. 490.

3:30–4:30 P.M. Section Business—Room 2, Morrill Hall

Wednesday, June 25

9:00–12:00 A.M. Auditorium, Fleming Museum

O. J. HILL, *Chairman*

Symposium—Mastitis

Joint session of Extension and Production Sections

New developments in physiology and biochemistry of lactation. W. E. Petersen, University of Minnesota.

Observations in mastitis control problems. George E. Taylor, New Jersey College of Agriculture.

Mastitis a state centralized testing service. C. S. Bryan, R. E. Horwood and J. G. Hays, Michigan State College.

The development and use of the Whiteside test in mastitis control. James S. Murphy, New Jersey Experiment Station.

Panel Discussion

Herd Health Committee Report, Geo. E. Taylor, New Jersey, Discussion Leader; C. G. Bradt, Cornell University; J. G. Hays, Michigan State College; Ramer F. Leighton, University of Minnesota; Claude J. Fawcett, Massachusetts State College; A. I. Mann, University of Connecticut; and James S. Murphy, New Jersey State College.

1:30–4:30 P.M. Room 2, Morrill Hall

C. G. CUSHMAN, *Chairman*

E8—Roughage investigation by the Bureau of Dairy Industry. T. E. Woodward and J. R. Dawson, Bureau of Dairy Industry. 491.

E9—Feeding committee report. C. G. Cushman, Clemson Agr'l College.

E10—4-H Club committee report. H. A. Willman, Cornell University.

* Numbers following the titles refer to the page on which the abstract appears.

Panel Discussion

Effectiveness of roughages upon the economy of milk production. A. C. Baltzer, Michigan State College; Otto J. Hill, State College of Washington; L. A. Higgins, Mississippi A. & M. College; and E. H. Loveland, University of Vermont.

Thursday, June 26

9:00–11:45 A.M. Room 1, Morrill Hall

FLOYD JOHNSON, *Chairman*

Symposium—The display of extension teaching ideas

1. South Dakota—D.H.I.A. production and feed summary.
2. Minnesota—Record of D.H.I.A. identification.
3. Iowa—Visual aids of balancing rations.
4. Wisconsin—Method of scoring fieldmen in permanent record project.
5. Kansas—
6. Tennessee—
7. Mississippi—
8. Texas—
9. New Hampshire—
10. New Jersey—
11. New York—
12. Massachusetts—
13. Connecticut—
14. Vermont—
15. Other states—

E11—A study of the growth of 4-H dairy heifers and the reliability of heart girth measurements as a means of estimating live weight. H. W. Willman and G. W. Salisbury, Cornell University. 492.*

E12—Junior program designed to supplement the general adult dairy quality improvement program. Evert Wallenfeldt, University of Wisconsin. 493.

Thursday, June 26

1:30–3:30 P.M. Room 2, Morrill Hall

O. J. HILL, *Chairman*

Papers and Reports

E13—Milk and cream loss prevention for 4-H Club members. M. L. Flack, University of Nebraska. 495.

E14—Dairy quality improvement program for Wisconsin. Dave Nusbaum, University of Wisconsin. 496.

E15—Methods of combating the stockyard bull racket. Leland Lamb, American Dairy Cattle Club.

* Numbers following the titles refer to the page on which the abstract appears.

E16—Federated Artificial Breeding Association of New York. Stanley J. Brownell, Cornell University.
E17—Quality committee report. T. J. Jensen, Michigan State College.
E18—Testing committee report. C. R. Gearhart, Pennsylvania State College.
E19—Report type classification committee. James W. Linn, Kansas State College.
E20—Sire committee report. E. J. Perry, New Jersey State College.
3:30–5:00 P.M. General Business Meeting—Auditorium, Fleming Museum

PRODUCTION SECTION

Monday, June 23

3:00–5:00 P.M. Tour of Barns and Herds—Leave from Morrill Hall.

Tuesday, June 24

1:30–4:30 P.M. See Divisions A and B.

Wednesday, June 25

9:00–11:00 A.M. Symposium—Mastitis, (See Extension Section), Auditorium, Fleming Museum.
1:30–4:30 P.M. See Divisions A and B.

Thursday, June 26

9:00–11:00 A.M. Auditorium, Fleming Museum

T. E. WOODWARD, *Chairman*

Symposium—Roughages

Making greater use of forage. T. E. Woodward, U. S. Bureau of Dairy Industry. (discussion)
The economics of grassland farming. C. B. Bender, New Jersey Agricultural College. (discussion)
Under what conditions and to what extent can grass silage profitably replace hay or corn silage in the rations of dairy cows? F. B. Morrison, Cornell University. (discussion)
Dairy farming without corn. H. A. Herman, University of Missouri. (discussion)

1:30–3:30 P.M. Curricula Symposium—Auditorium, Fleming Museum.
3:30–5:00 P.M. General Business Session—Auditorium, Fleming Museum.

PRODUCTION SECTION—DIVISION A

Tuesday, June 24

W. E. Petersen, *Chairman*

1:30–3:30 P.M. Room 27, Williams Science Hall

Milk Secretion

P1—The hormonal preparation of rats for lactation. R. P. Reece, New Jersey Agricultural Experiment Station. 497.*

P2—The effect of thyroprive goat's milk on experimental hyperthyroidism. J. W. Hibbs, T. S. Sutton and W. E. Krauss, Ohio Agricultural Experiment Station. 498.

P3—The effect of thyroxine on the lactogenic hormone in the urine of dairy goats. Victor Hurst, Joseph Meites and C. W. Turner, Missouri Agricultural Experiment Station. 499.

P4—The effect of diethystilbestrol on milk secretion. Arless Spielman, L. M. Ludwick and W. E. Petersen, University of Minnesota. 499.

P5—Anatomy and physiology of the teat sphincter. Dwight Espe and C. Y. Cannon, Iowa State College. 500.

P6—A comparison of the utilization of β-hydroxybutyric acid, glucose and oxygen by the mammary gland of the normal and ketosis cow. J. C. Shaw, University of Connecticut. 500.

P7—The effect of glucose feeding upon the concentration of acetone bodies in blood and urine and upon the milk and milk fat produced in the normal bovine. C. B. Knodt, University of Connecticut. 501.

P8—The effect of ketosis and glucose therapy in ketosis upon milk fat synthesis. J. C. Shaw, University of Connecticut. 502.

P9—A study of normal variations of acetone bodies in the blood and urine of dairy cattle. C. B. Knodt, University of Connecticut. 502.

3:30–4:30 P.M. Section Business—Room 27, Williams Science Hall

Wednesday, June 25

1:30–3:30 P.M. Room 27, Williams Science Hall

Milk Secretion and Reproduction

P10—Glucose therapy in ketosis in cattle. J. C. Shaw and Ross C. Powell, Jr. University of Connecticut. 503.

P11—Progress report on the relation of the ration to the composition of milk. E. B. Powell, Ralston Purina Company. 504.

P12—The influence of frequency of milking on milk production. L. M. Ludwick, Arless Spielman and W. E. Petersen, University of Minnesota. 505.

* Numbers at the end of the title refers to the page number on which the abstract appears.

P13—The chlorine tolerance of certain mastitis bacteria. R. K. Waugh, P. R. Elliker, J. H. Hilton and J. F. Bullard, Purdue Agricultural Experiment Station. 506.

P14—The influence of oat juice extract upon the age of sexual maturity in rats. E. T. Gomez, A. M. Hartman and L. P. Dryden, Bureau of Dairy Industry. 507.

P15—The effect of a high and low protein ration on the gonadotropic content of male rat pituitaries. E. G. Weatherby and R. P. Reece, New Jersey Agricultural Experiment Station. 508.

P16—The evaluation of fertility in dairy bull semen. H. A. Herman and Eric Swanson, University of Missouri. 508.

P17—The effect of exercise on the amount and quality of a dairy bull's semen. O. L. Lepard, C. Edmund Shuart and Arden Foster, New Jersey Agricultural Experiment Station. 509.

P18—Some factors influencing the reproductive efficiency of Louisiana herds. D. M. Seath and C. H. Staples, Louisiana State University. 510.

3:30–4:30 P.M. Section Business—Room 27, Williams Science Hall

PRODUCTION SECTION—DIVISION B

Tuesday, June 24

H. A. HERMAN, *Chairman*

1:30–3:30 P.M. Room 11, Williams Science Hall

Herd Management and Feeding

P19—Progress report on a roughage program in herd management. C. B. Bender, New Jersey Agricultural Experiment Station. 510.

P20—Approved Ayrshire sire program. C. T. Conklin, Ayrshire Breeders' Association. 511.

P21—Some chemical determinations useful in silage studies. A. E. Perkins, Ohio Agricultural Experiment Station. 512.

P22—Corn meal as a grass silage preservative. G. Bohstedt, W. H. Peterson and G. P. Bahler, University of Wisconsin. 513.

P23—Trench silos for preserving cereals treated with molasses or phosphoric acid. H. A. Herman, A. C. Ragsdale and Warren Heathman, University of Missouri. 514.

P24—Calculating pasture yields with dairy heifers as experimental animals. H. B. Morrison and Fordyce Ely, University of Kentucky. 515.

P25—A study of the relationship of fat content in the dairy grain ration to milk and butterfat production. C. F. Monroe and W. E. Krauss, Ohio Agricultural Experiment Station. 516.

P26—The influence of sustained high fat intake upon milk fat production. N. N. Allen and J. B. Fitch, University of Minnesota. 516.

P27—The relation of mineral intake and sunshine to the vitamin D deficiency of mature dairy cattle. G. C. Wallis, South Dakota Agricultural Experiment Station. 517.

3:30–4:30 P.M. Section Business with Division A—Room 27 Williams Science Hall.

Wednesday, June 25

1:30–3:30 P.M. Room 11, Williams Science Hall

Vitamins and Minerals

P28—Observations on the quantitative requirement of mature dairy cattle for vitamin D. G. C. Wallis, South Dakota Agricultural Experiment Station. 518.

P29—Relation of skeletal reserves of calcium and phosphorus laid down during growth to persistence of milk production of dairy cows. L. S. Palmer and T. W. Gullickson, University of Minnesota. 519.

P30—The effect of feeding chloretone (trichlorobutyl alcohol) on the blood plasma ascorbic acid of dairy cattle. A. L. Bortree, E. C. Scheidenhelm and C. F. Huffman, Michigan State College. 520.

P31—Some observations on the carotene content of the blood plasma of dairy cows. Harold Goss and S. W. Mead, University of California. 521.

P32—Vitamin A levels in the blood plasma of dairy cattle on winter rations and the influence of vitamin A supplementation on certain constituents of the blood. Paul H. Phillips, P. D. Boyer, H. A. Lardy and N. S. Lundquist, University of Wisconsin. 522.

P33—The blood plasma vitamin A content of the newborn calf and its relation to certain calfhood diseases. Paul H. Phillips, N. S. Lundquist and Paul Boyer, University of Wisconsin. 522.

P34—The carotene (provitamin A) requirements of dairy cattle for lactation. A. H. Kuhlman and W. D. Gallup, Oklahoma A. and M. College. 522.

P35—Further studies of the effects of vitamin A deficiency on reproduction. S. L. Hansard and T. S. Sutton, Ohio State University and Agricultural Experiment Station. 523.

P36—Some ocular changes and deficiency manifestations in mature cows fed a ration deficient in vitamin A. L. A. Moore, Michigan State College. 524.

3:30–4:30 P.M. Section Business with Division A—Room 27, Williams Science Hall.

MANUFACTURING SECTION

Monday, June 23

2:00–4:00 P.M. Dairy Laboratory, Morrill Hall

Judging of Dairy Products

Tuesday, June 24

E. S. GUTHRIE, *Chairman*

1:30–3:30 P.M. Auditorium, Fleming Museum

Symposium—Properties and Utilization of Cream

Industrial problems. J. L. Hileman, Dairymen's League Co-operative Association, Inc.

Production of cream on farms and in plants. C. H. Parsons, Swift and Company.

Flavor. G. Malcolm Trout, Michigan State College.

Viscosity. Paul F. Sharp, Cornell University.

Whipping. H. H. Sommer, University of Wisconsin.

Freezing and storage. F. M. Scales, Sheffield Farms Company.

Reconstitution and utilization. R. Whitaker, Sealtest, Inc.

Churning. Emerson W. Bird, Iowa State College.

3:30–4:30 P.M. Section Business. Auditorium, Fleming Museum.

Wednesday, June 25

9:00–11:00 A.M. See Divisions A and B.

Wednesday, June 25

H. F. JUDKINS, *Chairman*

1:30–3:30 P.M. Auditorium, Fleming Muesum

Symposium—Milk Pasteurization

Engineering principles. G. W. Putnam, Creamery Package Mfg. Co.
Loomis Burrell, Cherry-Burrell Corporation.

Time-temperature inter-relationships in milk pasteurization. A. C. Dahlberg, New York (Geneva) Agricultural Experiment Station.

High-temperature pasteurization. G. C. Supplee, Borden Biological and Chemical Research Laboratories.

Control of thermoduric organisms. A. C. Fay, H. P. Hood & Sons.

Equipment sanitation from the health official's viewpoint. W. D. Tiedeman, New York State Department of Health.

The Public health aspect of quality control. Sol Pincus, New York City Department of Health.

The industrial aspect of quality control. M. E. Parker, Beatrice Creamery Company.

3:30–4:30 P.M. Section Business. Auditorium, Fleming Museum.

Thursday, June 26

9:00–11:00 A.M. See Division A and B.

1:30– 3:30 P.M. Curricula Symposium. Auditorium, Fleming Museum.

3:30– 5:00 P.M. General Business Session. Auditorium, Fleming Museum.

MANUFACTURING SECTION—DIVISION A

Wednesday, June 25

C. D. Dahle, *Chairman*
9:00–11:00 A.M. Room 27, Williams Science Hall

Bacteriology

M1—The role of acid cleaning agents in dairy detergency. Milton E. Parker and G. W. Shadwick, Jr., Beatrice Creamery Company. 525.*

M2—The value of acidifying milk and cream cans from the standpoint of the effect upon quality. Alvin Rippen and L. H. Burgwald, Ohio State University. 525.

M3—The bacteriological spoilage of milk held near the freezing point. J. M. Sherman, G. M. Cameron and J. C. White, Cornell University. 526.

M4—Thermoduric bacteria in milk. III. The effect of changing agar and temperature of incubation for plate counts on the problem of thermoduric bacteria in milk. J. L. Hileman, Clarence Moss and Betty Stead, Dairymen's League Cooperative Association. 527.

M5—Effect of growth of *Pseudomonas putrefaciens* on aroma compounds in butter. P. R. Elliker and B. E. Horrall, Purdue University. 528.

M6—The effect of *Streptococcus agalactiae* upon the standard plate count of milk. Max E. Morgan and E. O. Anderson, University of Connecticut. 528.

M7—The lethal effectiveness of ultra-violet rays applied to milk. G. C. Supplee, G. E. Flanigan and O. G. Jensen, Borden Biological & Chemical Research Laboratories. 529.

M8—Bacteriological problems in short-time, high-temperature pasteurization. Harold Wainess, York Ice Machinery Corp. 530.

Thursday, June 26

9:00–11:00 A.M. Room 27, Williams Science Hall

Dairy Chemistry

M9—The foaming of milk and certain milk products in relation to their surface-active constituents. M. S. El-Rafey and G. A. Richardson, University of California. 530.

M10—Factors affecting the gas content of milk. C. I. Noll and G. C. Supplee, Borden Biological & Chemical Research Laboratories. 531.

M11—Factors influencing the response of cream to a rebodying process. F. M. Skelton and H. H. Sommer, University of Wisconsin. 532.

* Numbers following the titles refer to the page number on which the abstract appears.

M12—An improved micro-Kjeldahl apparatus and procedure for the analysis of milk. M. C. Rhees, T. R. Freeman and Chas. N. Shepardson, A. & M. College of Texas. 533.

M13—A progress report on the utilization of apple products, especially apple syrups and juices, in producing soft-curd milk. C. C. Flora and C. W. Holdaway, Virginia Agricultural Experiment Station. 534.

M14—The determination of citric acid in milk by the pentabromo-acetone method. E. F. Deysher and George E. Holm, Bureau of Dairy Industry. 534.

M15—The effect of flash forewarming upon the heat stability of evaporated milk. B. H. Webb and R. W. Bell, Bureau of Dairy Industry. 535.

11:00–12:00 A.M. Section Business. Room 27, Williams Science Hall.

MANUFACTURING SECTION—DIVISION B

Wednesday, June 25

L. H. BURGWALD, *Chairman*

9:00–11:00 A.M. Room 11, Williams Science Hall

Ice Cream and Milk

M16—The influence of homogenizing pressures on the "dryness" of ice cream when drawn from the freezer. J. H. Erb and John Whitworth, Ohio State University. 535.

M17—Monoglyceride-gelatin as an ice cream stabilizer. P. S. Lucas, Michigan State College. 536.

M18—A method for the preparation of acid casein for use in ice cream. L. P. Teichert, T. R. Freeman, W. S. Arbuckle and Chas. N. Shepardson, A. & M. College of Texas. 537.

M19—The temperature method for control of whipping in ice cream. Alan Leighton, Bureau of Dairy Industry. 538.

M20—Motion pictures as a medium for the study of ice cream. W. H. E. Reid, C. W. Decker, L. E. Smith, K. R. Minert, W. S. Arbuckle, and Joe Edmondson, Missouri Agricultural Experiment Station. 538.

M21—Homogenization index as calculated from measurements of fat globule size. A. W. Farrall, C. C. Walts and R. L. Hanson, Creamery Package Manufacturing Company. 539.

M22—The effects of the direct addition of carotene and mixed tocopherols on the development of oxidized flavor in milk. Edwin B. Williams and L. H. Burgwald, Ohio State University. 539.

M23—The influence of treated fibre milk containers on the incidence of copper-induced and sunshine oxidized flavors of milk. C. L. Roadhouse and J. L. Henderson, University of California. 540.

M24—An electric laboratory pasteurizer. H. B. Henderson, Thos. B. Harrison, C. E. Wylie, and H. A. Arnold, University of Tennessee. 541.

M25—Observations regarding the occurrence of oxidized flavor in milk from individual cows. H. B. Henderson, W. W. Overcast, and C. E. Wylie, University of Tennessee. 541.

M26—A small electric holder type pasteurizer. C. W. England, Arthur P. Wiedemer, and George J. Burkhardt, Maryland Agricultural Experiment Station. 542.

Thursday, June 26

9:00–11:00 A.M. Room 11, Williams Science Hall

Cheese and Butter

M27—Some factors influencing the quality of cream cheese. B. M. Zakariasen and W. B. Combs, University of Minnesota. 543.

M28—A short method of making a soft cheese similar to cream cheese. E. L. Reichart and L. K. Crowe, University of Nebraska. 544.

M29—A survey of commercial cottage cheese. Milton J. Foter, E. O. Anderson and L. R. Dowd, University of Connecticut. 544.

M30—The relationship of acidity to the quality of American Cheddar cheese. H. L. Wilson, S. A. Hall, and H. R. Lochry, Bureau of Dairy Industry. 545.

M31—Keeping quality of butter stored at low temperature for six years. B. J. Scheib, E. S. Guthrie, and C. N. Stark, Cornell University. 545.

M32—Mold mycelia in cream. E. R. Garrison and J. H. Gholson, Missouri Agricultural Experiment Station. 546.

M33—The effect of udder infection and late lactation on the methylene blue-borax test for mold mycelia in cream. E. R. Garrison and J. H. Gholson, Missouri Agricultural Experiment Station. 547.

M34—The effect of various factors on mold mycelia in cream and butter. W. H. E. Reid, Joe Edmondson, and W. S. Arbuckle, Missouri Agricultural Experiment Station. 548.

1:30–3:30 P.M. Auditorium, Fleming Museum

H. B. ELLENBERGER, *Chairman*

Symposium-Curricula

ABSTRACTS OF PAPERS

EXTENSION SECTION

E1. Supervisory Standards for State Men in Charge of D.H.I.A. Work.
R. G. Connelly and R. W. Dickson, Virginia Polytechnic Institute.

Dairy herd improvement association records are used in compiling proved-sire records which are accepted and used widely as a basis for dairy cattle improvement; therefore, it is important that minimum standards of supervision be established to insure the accuracy and reliability of all dairy herd improvement association records regardless of the association or state in which they are obtained.

Minimum standards of tester training and the supervision he receives to insure accurate and reliable records may be listed as follows:

A. Qualification of Tester.
  1. Farm experience, preferably dairy farm experience.
  2. Training.
    a. High School graduate with Vocational Agriculture training.
    b. Satisfactorily completed D.H.I.A. tester training short course of not less than four weeks, preferably six weeks. Short course to be conducted by State Agricultural College.
    c. Licensed by State to conduct Babcock test.

B. Supervision of Tester.
  1. Field Supervision.
    a. Coach each new tester in the testing and record work of at least two herds.
    b. Inspection of testing equipment annually, for completeness and working condition.
    c. Report by tester to county agent monthly and present association monthly summary report.
    d. Visit tester at least twice a year to observe his work. The county agent or extension dairyman may make visits.
    e. Hold semi-annual conferences with at least twenty per cent of association membership to check quality of service rendered by tester.
    f. Examine at least four record books selected at random from each association to see that records are complete in data recorded and calculations accurate.
  2. Conferences.
    a. Annual, preferably semi-annual, regional conferences held with testers by extension dairymen where testing problems

and methods are discussed to acquaint testers with current information on up-to-date testing work.

b. Individual semi-annual conferences with testers by extension dairyman to discuss problems of D.H.I.A. work and to correct inefficiencies of the tester.

C. OFFICE RECORDS.

1. Testers to submit monthly barn sheets to state office. Barn sheets to be used as a check on the monthly work of the tester and as a permanent file.
2. State office to disperse ear tags to testers and to keep permanent record of B.D.I. 717 and B.D.I. 718 reports.
3. State office to prepare annual statistical summaries for annual meeting of each organized association.

E4. Survey of Modified Testing. Summary of Report for the Testing Committee. E. H. LOVELAND, University of Vermont.

Reports were received from 28 states relative to modifications in method of conducting dairy test record work with farmers. Seven states report "owner-sampler" plan where samples of milk taken by the owner are tested cooperatively, either through a regular D.H.I. association or through an organization set up for that purpose. Four report favorably and three report unfavorably on this plan. Five states report bi-monthly testing as a modified form of regular D.H.I. work. Three report favorably, one unfavorably, and the other undecided. Seven states report no modifications, with the remainder minor modifications.

In Vermont, the bi-monthly system of testing has been in successful operation for over a year with seven associations sponsored by creamery organizations. On January 1, 1941, these associations had 301 herds with 6289 cows on test. Up to January 1, 1941, six associations had completed their first year of testing with an average production of 227 lbs. fat per cow against a state D.H.I.A. average of 291 lbs. for the monthly associations. These bi-monthly associations are apparently reaching many average dairymen who never had, and probably never would pay the price for membership in a regular monthly association. One association has finished its second year with 70 per cent of the herds testing for two years showing improved production and efficiency, indicating that the bi-monthly form of testing was successful in herd improvement. A normal percentage of herds is continuing into the second and third year of testing.

A comparison of creamery deliveries with bi-monthly estimates shows the herd totals to be as accurate as those found in monthly associations. There is a marked tendency among the more interested members to shift to a monthly form of testing as soon as such service is available. Bi-monthly testing is not generally considered as satisfactory as monthly testing. The

bi-monthly association is recognized for publicity and records are accepted on the same basis as those of a monthly association. Because of greater length of time between visits, the bi-monthly association requires greater skill on the part of the tester to make adjustments in the records and to help the farmer in herd improvement.

E5. Method of Reporting Kinds and Quality of Feed in Dairy Herd Improvement Association Work. A. J. CRAMER, University of Wisconsin.

Feeding records are only as valuable as they are complete. Herd owners should cooperate with the fieldman and see that he weighs the feed at night as accurately and completely as possible for each cow. If the hay, silage, and grain are not weighed on each month's visit by the fieldman, the herd owner will not receive full value for the money he has spent in Association membership dues or fees.

Cows should be fed according to their individual production, to obtain the greatest returns for the feed consumed. The ultimate purpose of keeping complete feeding records is to enable the dairyman to improve his herd production through practicing intelligent and efficient feeding methods. On many farms, the feed cost of a cow represents about half of the total cost of keeping her. That's why its so important to obtain as nearly accurate feed weights as possible. The feed weights one day each month serve as a basis for charging the feed costs for the entire month.

Recommended practices for fieldmen are: *First,* the ration being fed should be recorded on the back inside cover of the member's herd record book. *Second,* the prices paid for purchased feeds, and the values of home-grown feeds are to be recorded in the back of the book. *Third,* the complete record of all feeds fed should be listed on the monthly herd summary page in the herd book. *Fourth,* the total pounds of hay, silage, and grain, and the per cent of protein is to be listed for each individual cow. *Fifth,* a barn-feeding chart called a feeder's guide is posted over the feed box listing the cows, the amount of butterfat each cow produced that month, and the recommended amount of grain each cow might receive each feeding according to her production. The results of feeding according to production in one herd showed a gain of 40 pounds daily in milk, or $18.00 higher returns over feed cost for that month.

At the close of the year, the fieldman will have the feed totals for each cow and the totals of all feeds on the entire herd. These feed weights are helpful in determining the amount of feed needed for the herd the next year.

The difference in quality of roughage and grain is reflected in the price. We aim to use uniform prices within a county for similar quality of feeds, using the price of home-grown feed at what it is worth on the farm. The purchase price is used on all feeds.

The time of purchase, the quantity, and the quality of feeds used are price determining factors.

If comparisons are made as to the production efficiency of herds, uniform prices for butterfat as well as for similar quality of feeds fed should be used.

The weak spot in our records is the lack of feed information gathered by the fieldman. It is important that fieldmen use care and exact methods in weighing all roughage and grain every month; reporting kinds, amounts, and quality of feed used in Dairy Herd Improvement Association work. Such information is valuable in building toward more efficient production records.

E6. New D. H. I. A. Conversion Factors—By Breeds. J. F. Kendrick, Bureau of Dairy Industry.

For some time it has been generally recognized that the age-conversion factors used in compiling dairy herd-improvement association proved-sire data for all breeds are not entirely satisfactory for converting production records of individual breeds of cows to a mature-equivalent basis.

Heretofore sufficient data were not available to justify a study of the relationship between age and production by breeds. Through the operation of the Nation-wide dairy herd-improvement association proved-sire program, however, the States have reported 305-day lactation records for permanent recording and data have accumulated in sufficient volume so that a study could be made and reliable age-conversion factors for each breed could be developed.

Such a study was made of the records of approximately 17,000 Ayrshire, 8,000 Brown Swiss, 64,000 Guernsey, 63,000 Holstein, and 60,000 Jersey cows. In one part of the study a straight tabulation of 305-day lactation records for cows of each breed for different ages was compiled. That is, the records of all cows 2 years of age were added and averaged, the records of all cows 3 years of age were added and averaged, and so on for the various ages. In another part of the study only the records of cows with two or more consecutive lactation records were used in an effort to determine the degree of slope of the production trend line from year to year and thereby tend to reduce the influence of culling.

New age-conversion factors, based on the results of this study, by breeds, are recommended for adoption by the American Dairy Science Association for use in dairy herd-improvement association proved-sire work.

E7. Uniform Annual Reports for Artificial Breeding Associations, Including Standardized Methods of Calculating Conception Rates. Ralph A. Corbett, University of Maine.

With more than twenty states having one or more Artificial Breeding Associations there is need for a uniform method of reporting activities and

progress of these associations on a state and national basis. This would assist those people now engaged in the successful operations of these associations to profit by the methods and results of all other associations.

There is need within every association for certain standard forms. These include: (1) Daily Bull Semen Report, (2) Breeding Receipt, (3) Farmers Cow Breeding Record, (4) Individual Cow Case Card (or equivalent), (5) Daily Bull Report of Conception Efficiency, (6) Yearly Annual Report, and (7) Simple but Complete Financial Report Forms.

At present there is a wide variation in the methods used in calculating conception rates which may influence decidedly the final success of the associations. These methods should be standardized within the state and nation, so that everyone will be measuring results comparable to all other results. For example: In associations where three services per cow are allowed, results may be calculated on two services as practically every man who has paid for three services will call for them. In many cases it may be difficult to determine what happens with the cow after three services—if conception is figured on three or more services. Where pregnancy examinations are not being carried out, if a cow does not show signs of oestrum for six weeks following breeding she may be considered in calf, although a small per cent will prove to be open.

Uniform Annual Reports would be especially helpful. They might include:

1. Number members enrolled.
2. Number members having cows inseminated during year.
3. Number different cows inseminated.
4. Number of total inseminations.
5. Number cows pregnant. Number failing to conceive.
6. Breeding ratio.
7. Number cows becoming pregnant on one, two, and three services.
8. Names and number of each bull used with the breeding record of each bull.

E8. Roughage Investigations by the Bureau of Dairy Industry. T. E. WOODWARD AND J. R. DAWSON, Bureau of Dairy Industry.

This is a review of the work done by the Bureau of Dairy Industry at Stations located in a number of states, together with a discussion of the practical implications of the work.

A total of 134 year records on roughage alone has been obtained. There is a considerable number of other records made by supplementing the roughage with moderate amounts of grain. The production that can be expected when different methods of feeding are practiced is discussed, together with the conditions under which grain supplements will pay.

Some 150 to 175 lots of experimental silage, mostly with the hay crops,

have been made in the last few years, and these have been fed for considerable periods in a number of cases. The place of grass and legume silage in dairy farming is discussed.

The yield of different crops for pasture has been determined and methods of pasture management have been studied. The conditions under which rotation grazing and the use of annual and supplementary pastures are desirable are discussed.

The utilization of crops in the form of pasturage, hay or silage has been studied with reference to yields, feeding value, and loss of nutrients. This work is discussed with reference to its practical aspects.

E11. A Study of the Growth of 4-H Dairy Heifers and the Reliability of Heart Girth Measurements as a Means of Estimating Liveweight. H. A. WILLMAN AND G. W. SALISBURY, Cornell University.

Few farms are equipped with scales suitable for weighing dairy cattle. Some dairymen have not realized how small their cattle appeared to others until they have lost a sale. In some sections of New York State where breeders depend to some extent on the income from the sale of surplus replacement cattle, buyers frequently offer the criticsm that the cows and young stock are too small.

It is apparent also that some 4–H members are not growing their yearling cattle too well. They have not been checking the growth or size of their cattle because scales are not available. Because of this, an accurate or at least some practical means of estimating weights and determining gains would be useful. Likewise, the regular and frequent use of the tape should help in stimulating and maintaining the interest of the 4–H member in the proper development of his cattle.

Several investigators already have demonstrated that a relationship exists between heart girth measurements and liveweight. Kendrick and Parker[1] of the Bureau of Dairy Industry, United States Department of Agriculture and Ragsdale[2] of the Missouri Station have published tables for estimating the live weights of cattle from heart girth measurements.

From these data tape measures have been developed, on one side of which there appears the measurements in inches and on the other side the estimated weights.

Because of the ease with which the heart girth measurement can be taken, it seemed desirable to study not only the relative growth or size of 4–H heifers but to determine how accurate an estimate of body weight could be obtained on a group of 4–H calves and heifers by means of the tape in common use. Apparently no one else has reported on a study of this sort,

[1] Bureau of Dairy Industry, Bull. 695, 1936.

[2] Missouri Bull. 354.

especially on heifers of widely different breeding which had been raised on many different farms and fed by different feeders under varying conditions.

The objects of study were:

1. To determine whether 4-H club boys and girls are growing their young stock satisfactorily.
2. To determine the reliability of heart girth measurement[1] weight estimates as compared with actual scale weights for each breed.
3. To determine the direction of error in estimating weights by the tape[1] if differences occur.

A study of data on 126 head of unselected 4-H cattle ranging in age from 5 to 35 months which were exhibited at the 1940 New York State Fair indicated that:

1. Most of the 4-H heifers of the younger ages were heavier than the suggested standard weights[3] while the older heifers often weighed less than the standard.
2. The weight tape[1] in common use in the state is more accurate in estimating the weights of Holstein heifers than in estimating the weights of Ayrshires, Guernseys, and Jerseys.
3. The tape weight estimates on calves of the smaller breeds were significantly higher than the actual weights when studied statistically.
4. No evidence was obtained to indicate that the small variation in the condition of the heifers influenced the estimate of liveweight.

E12. A Junior Program Designed to Supplement the General Adult Dairy Quality Improvement Program. Evert Wallenfeldt, University of Wisconsin.

Junior groups who have acquired "dairy sanitation consciousness" have often remedied faulty practices which milk inspectors and dairy fieldmen have sometimes failed to accomplish by working with adults alone. In order to take advantage of this factor to the greatest extent feasible, the Wisconsin dairy industry extension service has carried on a broad junior extension program for dairy quality improvement. The aim has been to supplement and not to replace in any way the general adult dairy quality improvement program which has been carried on for many years.

The success of such a program depends on the development of dairy sanitation consciousness and practical applications on the part of local agricultural and home economics leaders as well as on the part of the boys and girls participating.

Some of the tools used extensively are: (1) dairy products judging contests, (2) dairy demonstration contests, (3) dairy exhibit contests and (4) special project activities such as construction of cooling tanks and milk

[3] Morrison, F. B., Feeds and Feeding, 20th ed., p. 615.

houses, cow clipping rings and methylene blue and sediment testing, etc. A large number of voluntary local leaders have been enlisted to help with this work.

The junior dairy products judging work consists of several separate phases. (1) 4–H Club dairy products judging schools and contests, (2) F.F.A. and F.H.A. dairy products judging schools for teachers and contests for members, (3) rural school teachers judging schools and contests for rural school pupils in upper grades.

The 4–H Club phase includes: (1) a number of district dairy products judging training meetings (12 during 1941) for both 4–H leaders and members, (2) the conducting of county contests, (3) assisting county agents in the procuring and scoring of samples for local contests, and (4) a state 4–H Club dairy products judging contest at the Junior State Fair. Dairy products are now also included in the Junior Fair foods judging contests which involves the foods and nutrition project members from practically all counties in the state.

The F.F.A. dairy products judging work done by the extension specialists is covered largely by the following: (1) district dairy products judging schools for agricultural teachers (five were held during 1941), (2) assistance with subject matter material and the scoring of products for local judging contests where feasible. The F.F.A. dairy products judging is climaxed by a statewide F.F.A. dairy products judging contest which is conducted by the resident staff of the department of dairy industry and the specialists.

The F.H.A. dairy products judging was started by a dairy products judging meeting for all the high school rural home-making instructors at their state conference. This was followed with district dairy products judging training schools for all of these home economics teachers (five during 1941).

Dairy products judging training meetings for rural district school teachers have been conducted by the specialists. Contests for rural school contestants have also been held but it is expected that the county agents and high school teachers will handle these themselves in the future.

The dairy demonstration program consists of (1) county and district demonstration training meetings for 4–H leaders and members, (2) district demonstration training meetings, (3) six district dairy demonstration contests and a final state contest at the Junior State Fair for the district winners in each of the two divisions, dairy production and dairy foods.

The demonstration phase of the junior program reached the greatest number of people in that the 650 demonstrators participating gave their demonstrations before audiences totaling about 84,500 people in over one-half of the counties, but the dairy products judging program has been carried into every county of the state.

E13. **Milk and Cream Loss Prevention for 4-H Club Members.** M. L. FLACK, University of Nebraska.

During the spring of 1940 the State Quality Committee and butter manufacturers working with the extension service of the Agricultural College put into operation a "Milk and Cream Loss Prevention" project. This project became a part of the state 4-H Club program where all 4-H Club members regardless of the type of work carried could compete.

In the past, considerable work has been done in Nebraska on quality improvement of dairy products; but none of them seemed to attract as much attention or receive as much favorable comment as the "Milk and Cream Loss Prevention" did the past year.

It was the intention of the State Committee on quality as well as dairy manufacturers to center attention on the improvement needed on the farm around the dairy farm buildings with special emphasis on the importance of destroying weeds that cause off-flavors in milk and cream.

In the middle west weeds have become a terrible menance to the dairy industry. This is especially true where pastures have been killed out by the long continued drouth. It has been estimated by the dairy industry that the farmers of the middle west have lost annually thousands of dollars from poor or low quality cream caused by weeds in the pastures, the worst offender being pennycress, or stink weed.

The Quality Committee and butter manufacturers working through the 4-H Club department made available rather liberal awards for five different kinds of competition in this "Milk and Cream Loss Prevention" work. Around 23,000 Club members learned about this work and awards offered through their County Agriculture Agent and 4-H Club leaders.

Money awards were offered for the following competition:

1. Awards for 4-H Club team demonstrations portraying new and better methods of handling milk and cream for its improvement. These teams in order to qualify for the award had to put on their demonstration at least three times in their home community and at County and State Fairs.
2. Awards for Posters dealing with the elimination of milk and cream losses. These posters to be displayed at County and State Fairs.
3. Awards for news articles written for local and state press on "How to Eliminate Losses on Milk and Cream" caused by contamination with weeds and other foreign material.
4. Awards for booths at County and State Fairs setting out approved methods of pasture management and other measures for improving milk and cream on the farm.
5. Awards for the Clubs as groups where they cooperated in destroying weeds in their community as well as cleaning barn yard. This to be done by mowing and burning the weeds wherever found.

The State Quality Committee as well as the 4-H Club and Extension Departments were well satisfied with the results received this past year. A number of very effective demonstrations were put on in communities and at the State Fair. One demonstration team demonstrated before more than 3,000 people in different communities. One 4-H Dairy Club in the state was responsible for the destruction of 700 acres of weeds in their community. A number of good news articles were prepared and published in various newspapers and farm papers in the state. Several very effective booths were exhibited during the State Fair, and at many County Fairs. One leader made the remark that his club through these contests had made their whole community weed conscious and that many of the farmers were taking steps to rid their farms of this pest.

E14. The Dairy Quality Improvement Program in Wisconsin. DAVE NUSBAUM, University of Wisconsin.

The Department of Agriculture and the College of Agriculture in Wisconsin have jointly adopted a quality milk program which is put on in the state on a county by county basis and now covers nearly half of the state.

The program consists largely of the education and development of quality consciousness on the part of both milk producers and plant operators.

County Agricultural Agents are key men in the program. Meetings which are conducted by farmers are held in every schoolhouse in a county at the beginning of the program. All dairy plants in the county grade milk by the methylene blue test and the sediment test and cream by taste and smell. The results of these tests are sent to the farmers by the plant. The college specialists and the county agent are largely responsible for the educational phases of the program—the Department of Agriculture handles the regulatory end of the program.

As a result of this work, many plants in the state are adopting systems of paying for milk and cream by grade.

The standards which are used in the program would be applicable to other sections of the country.

Manufacturing records of many plants in the state indicate a definite improvement in the quality of manufactured products. This frequently has resulted in an increase in price received or in the establishment of a better market.

This program is set up primarily for cheese, butter, and evaporated milk sections in the state.

PRODUCTION SECTION

P1. The Hormonal Preparation of Rats for Lactation.* R. P. REECE, New Jersey Agricultural Experiment Station.

A study was made of the possibilities of preparing rats for lactation by hormonal administration. Forty-two experimental rats were used in this work.

When a normal rat mated, another sexually mature rat was started on hormonal treatment. In the majority of experimental rats injections were initiated when they were in estrus. Vaginal smears were made daily to determine if a prolonged luteal phase ensued. Two to three days following parturition in a normal rat the pups were transferred to an experimental rat. Litters were standardized at 6 and they were frequently observed in order to determine if they had nursed. Litter body weights were recorded daily. Some of the rats which did not raise their litters, were sacrificed and their mammary glands observed macroscopically to ascertain the degree of mammary development.

The results are summarized under the headings of hormonal treatment which the experimental rats received.

No treatment.—Four multiparous rats nursed but did not raise their litters.

Gonadotropic principle of pregnancy urine (Follutein).—Four multiparous rats failed to raise litters placed with them. The mammary glands of one rat were not well developed while the glands of the remaining 3 rats were well developed.

Follutein plus an estrogen (Progynon–B).—Six rats, 2 virginal and 4 multiparous, failed to raise the litters placed with them. There was no evidence of nursing in 5 of the 6 litters. The glands of 3 rats were not extensively developed.

Gonadotropic principle of pregnant mare serum (Gonadin).—Seven rats received Gonadin injections, but, litters were available for only 6 of the rats. One of the 6 rats raised 2 successive litters, the first litter averaged 52 grams at 21 days and the second litter averaged 34 grams at a similar age. Of the remaining 5 rats for which litters were available 2 did not show signs of maternal instinct. Although a litter was not available for the seventh rat milk could be expressed from the teats.

Gonadin plus Progynon–B.—Of 9 rats, 2 virginal and 7 multiparous, 7 failed to raise their litters. The average body weight at 21 days in one litter was 45 grams while in the second litter it was 40 grams. Maternal instinct was lacking in 6 of the 9 rats. The glands of one rat were extensively developed and filled with milk.

* Journal Series Paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

Progynon–B.—Of 12 rats injected with Progynon–B, 5 raised their litters and one raised 4 of 6 pups. The average body weights in grams of the pups at 21 days were: 37; 34; 32; 33; 51; and 36. One of the 12 rats failed to nurse her liter. The glands of 3 rats were examined. The glands of one rat were well developed and contained some milk, the glands of the second rat consisted mainly of an extensive duct system and contained a small amount of milk, while the glands from the third animal showed complete development and it was possible to express milk from the teats.

P2. The Effect of Thyroprive Goat's Milk on Experimental Hyperthyroidism. J. W. HIBBS, T. S. SUTTON, AND W. E. KRAUSS, Ohio Agricultural Experiment Station.

Clinical reports of the effectiveness of thyroprive goat's milk in the alleviation of the symptoms of hyperthyroidism prompted the present study.

Preliminary work in which the effects of normal goat's milk on the basal metabolic rate of rats were compared with that of milk from a thyroidectomized goat,[1] produced slight although erratic and unreliable evidence that thyroprive goat's milk effected a lowering of metabolic rate.

Since the clinical reports of the efficacy of thyroprive goat's milk were obtained by feeding such milk to hyperthyroid patients, it was considered advisable to study the effects on hyperthyroidism experimentally produced in rats.

Three groups of littermate male rats were maintained on a milk ration for a period of two weeks. Eleven animals were used in each group. Post absorptive metabolic rates were determined on the first, eighth and fifteenth day. Beginning on the ninth day and continuing through the fourteenth day each rat was treated with thyroxin, administered by subcutaneous injection at the rate of 0.2 mg. per 100 gm. body weight.

During the first week in which the milk was fed but no thyroxin injected, there was no significant change in metabolic rate. Minor inconsistencies may be explained by our inability to get a true basal due to activity of the animal. Several runs usually must be made before the animal becomes accustomed to the apparatus.

Following thyroid treatment, metabolic rates rose sharply but not all to the same level. The B.M.R. of those receiving cow's milk increased an average of 82 per cent, those receiving normal goat's milk increased on the average of 85 per cent, while the average increase in B.M.R. of those receiving thyroprive goat's milk was 68 per cent. This difference of 14 per cent and 17 per cent between the metabolic rate of rats receiving thyroprive goat's milk and those receiving cow's or normal goat's milk, respectively, is

[1] We gratefully acknowledge the assistance by Dr. Harold Beeson who performed the thyroidectomy.

interpreted as significant evidence of the presence of an antithyroxin principle in thyroprive goat's milk.

P3. The Effect of Thyroxine on the Lactogenic Hormone in the Urine of Dairy Goats.* VICTOR HURST, JOSEPH MEITES, AND C. W. TURNER, Missouri Agricultural Experiment Station.

During the past few years numerous investigations have shown that the administration of thyroid tissue or thyroxine has stimulated increased milk secretion especially during the declining phase of lactation in dairy cattle and goats. A number of theories have been advanced to explain the mode of action of thyroxine in stimulating increased yield of milk. It is possible that its action is through increasing heart rate with an increased volume of blood flowing to the udder. There follows also an increased cellular activity as shown by the increased metabolic rate, thus the epithelial cells of the mammary gland might be stimulated to a more rapid rate of milk synthesis. Thyroxine might increase the availability of the precursors of milk in the blood resulting in a greater uptake by the udder. There is also the possibility that one or more pituitary hormones which influence lactation might be secreted in greater amount.

As a method for the extraction and assay of the lactogenic hormone in the urine has been developed in our laboratory, it seemed of interest to compare the concentration of lactogen before and after thyroxine administration to lactating goats. The urine used for extraction was collected for 2 days prior to and 5 to 6 days following injection during which time the maximum rise in milk yield occurs.

While the work is still in progress and there may be variations to report later, there is at present no indication of a significant increase in the amount of lactogenic hormone in the urine of goats following thyroxine injection even with a material increase in the level of milk production.

P4. Effect of Diethylstilbestrol on Milk Secretion.† ARLESS SPIELMAN, L. M. LUDWICK AND W. E. PETERSEN, University of Minnesota.

Discovery of the estrogenic activity of diethylstilbestrol by Dodds, Goldberg, Lawson and Robinson has greatly facilitated the study of estrogen function in milk secretion.

This preliminary report deals with the effects of artificial administration of diethylstilbestrol on milk secretion. At this writing four lactating cows have been under observation for thirty days. Various amounts of diethylstilbestrol in cottonseed oil ranging from 10 to 100 milligrams have been injected intramuscularly at irregular intervals.

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 739.

† The diethylstilbestrol was furnished by Merck and Co.

Results to date have shown a marked increase in the butterfat and lactose percentage by 1 to 1.5 with no apparent effect on total milk production. Total protein and casein protein have not changed. A striking corollary is the marked rise of 100 or more mg. per cent in the level of blood fat and a significant increase in blood sugar. The increase in butterfat percentage began to decline about 48 hours after terminating the injections and returned to normal within five days. The increase in lactose secretion has persisted longer, as has the increase in blood fat and blood sugar.

The amount of diethylstilbestrol necessary to give the above results seems to be about 50 to 150 mg. for the cows used. The latent period has been about three days.

These results substantiate the report by Folley and Watson that diethylstilbestrol produced a prolonged increase in both the fat and lactose content of milk.

P5. Anatomy and Physiology of the Teat Sphincter. DWIGHT ESPE AND C. Y. CANNON, Iowa State College.

X-ray pictures give no indication that any vacuum develops at the end of the teat when the pressure exerted in milking is released. The length and tone of the teat sphincter may be of importance in preventing bacteria from entering the mammary gland.

P6. A Comparison of the Utilization of β-hydroxybutyric Acid, Glucose and Oxygen by the Lactating Mammary Gland of the Normal and Ketosis Cow. J. C. SHAW, University of Connecticut.

Blood samples for arteriovenous difference work were drawn simultaneously from the internal iliac artery and the subcutaneous abdominal mammary vein. The data to be presented include only those analyses made on bloods undergoing no blood concentration changes in the mammary gland. In fifteen experiments on normal cows there was a mean utilization of 1.83 $\pm$ (S.E.) 0.246 mg. per cent β-hydroxybutyric acid and 4.70 volumes per cent oxygen. In six arteriovenous differences in ketosis where the total blood acetone bodies exceeded 15 mg. per cent there was a mean utilization of 3.91 mg. per cent β-hydroxybutyric acid and 3.60 volumes per cent oxygen. The normal arteriovenous glucose difference for 40 cows was 9.3 mg. per cent. In 9 arteriovenous differences on ketosis cows with arterial blood sugar values below 35 mg. per cent there was a mean utilization of 9.5 mg. per cent. The failure of the gland to utilize more oxygen with the increase in the utilization of β-hydroxybutyric acid may be due to the use of this blood substance directly for synthesis. If, however, it is used by the gland for energy purposes the lack of an increase in oxygen utilization in ketosis appears to be best explained by a shift from a normal oxidation of other fat to an increased oxidation of β-hydroxybutyric acid. Due to the fact that most

tissues of the body apparently use β-hydroxybutyric acid for energy purposes, any hypothesis that this substance is used in actual synthesis must be accepted with reservation. It is difficult to explain, however, just why the gland should use only β-hydroxybutric acid for energy purposes since the other tissues apparently invariably use both β-hydroxybutyric acid and acetoacetic acid.

P7. The Effect of Glucose Feeding Upon the Concentration of Acetone Bodies in the Blood and Urine, and Upon the Milk and Milk Fat Produced in the Normal Bovine. C. B. KNODT, University of Connecticut.

The normal level of blood and urinary acetone bodies as well as the normal levels of milk and milk fat production were established prior to the glucose feeding period. The glucose was fed in the form of corn sugar three times per day in addition to the regular ration to the extent of six pounds per day for a ten day period to each of two cows; and six pounds per day for a period of twenty-five days to each of two other cows. Another cow was fed three pounds per day for a three day period, six pounds per day for the next three days, and this was increased to nine pounds per day for a period of four days. This cow received ten and one-half pounds per day for the next three days but on the evening of the third day she failed to consume all of the glucose fed but continued to consume six pounds per day for the next nine days.

After twenty-four hours of glucose feeding the concentration of blood and urinary acetone bodies had decreased 35.13 per cent and 23.46 per cent respectively. After three days of feeding, the concentration of blood and urinary acetone bodies had fallen 48.73 per cent and 54.08 per cent respectively. After ten days of continuous glucose feeding at a level of six pounds per day the concentration of blood and urinary acetone bodies was found to be 58.54 per cent and 52.62 per cent of the previously determined normal levels respectively. After twenty-five days of continuous glucose feeding at a level of six pounds per day the concentration of acetone bodies in the blood and urine was found to be 44.02 per cent and 60.00 per cent respectively of the established normals. Three days after the cessation of glucose feeding the levels were found to be 51.27 per cent and 45.92 per cent respectively in the blood and urine of the established normal concentrations. Six days after the cessation of feeding the blood and urinary acetone bodies were found to be 47.71 per cent and 56.55 per cent of the previously determined normal levels.

The average milk and milk fat production was 1.02 per cent and 10.37 per cent respectively below normal for the first ten days of feeding. The average milk and milk fat production for the twenty-five day feeding period was found to be 3.91 per cent and 10.05 per cent below normal respectively.

The milk and milk fat production for the six day period after the cessation of feeding was found to be 5.17 per cent and 3.50 per cent below normal respectively.

The saponification number, the Reichert-Meissl and Polenske values, and the iodine numbers were determined but no significant differences were found when the food intake was regulated so that the experimental animals did not refuse food. A fall in short chain fat acids was observed when large quantities of glucose were pumped into the rumen but is believed to be due to the refusal of food which followed.

P8. The Effect of Ketosis and Glucose Therapy in Ketosis upon Milk Fat Synthesis. J. C. SHAW, University of Connecticut.

In severe ketosis with the blood sugar and lactic acid low and acetone bodies high the short chain fatty acids of milk are quite low as shown by the various fat constants. In recovery in a four-week period with an increase in the blood sugar level from 20.9 mg. per cent to 44.9 mg. per cent the saponification number increased from 213.5 to 227.2, the Reichert-Meissl increased from 22.3 to 27.44 and the Polenske value increased from 1.49 to 2.90.

Pumping 6 pounds of glucose into the rumen of another cow by stomach tube resulted in 48 hours in an increase in glucose from 15.0 to 42.2 mg. per cent, the saponification number increased from 215.6 to 218.8, the Reichert-Meissl number increased from 25.4 to 28.9, the Polenske number increased slightly from 2.1 to 2.3. The iodine value showed a surprising increase from an already high value of 48.2 to 51.4. That the low blood glucose is not necessarily directly responsible for the abnormal fat constants is indicated by values obtained from the same cow three weeks later. At that time with a blood sugar value of 24.4 mg. per cent the saponification number was 228.0, the Reichert-Meissl number was 33.4, the Polenske number was 2.8 and the iodine number was 41.1. The cow was in much better condition clinically at that time and was eating fairly well although the blood acetone bodies were quite high. The milk fat constants for a number of ketosis cows during severe ketosis and following recovery will be presented.

P9. A Study of Normal Variations of Acetone Bodies in the Blood and Urine of Dairy Cattle. C. B. KNODT, University of Connecticut.

Various groups were set up composed of 12 mature cows, 9 twelve-month old heifers, 4 calves at birth, and 1 aged bull. Variations in relation to stage of lactation, stage of gestation, season of year, time of day, age, parturition, stage of oestrous cycle, total acetone body excretion for 8 to 24 hour periods, and effect of complete cessation of milking were studied.

All samples for blood and urinary acetone bodies were obtained when the animals were apparently normal in every respect. Analyses were deter-

mined immediately after samples were taken so as to minimize as much as possible the changes which occur. Blood was obtained from the jugular vein and potassium oxalate was used as the anticoagulant. Acetone bodies were determined by the method of Barnes and Wick (J. Biol. Chem., 131, 413, 1939).

Analyses on mature cows, calculated as acetone, consisted of 414 determinations for blood acetone and acetoacetic acid (Ave. 1.15 mgms. per cent), 436 determinations for total blood acetone bodies (Ave. 2.78 mgms. per cent, Min. .31, Max. 6.27), 369 determinations for urinary acetone and acetoacetic acid (Ave. 4.64 mgms. per cent), and 408 analyses for total urinary acetone bodies (Ave. 11.70 mgms. per cent, Min. .61, Max. 31.38).

A total of 64 analyses of non-pregnant heifers 12 to 22 months of age for blood acetone and acetoacetic acid (Ave. 0.91 mgms. per cent), for total blood acetone bodies (Ave. 2.03 mgms. per cent), urinary acetone and acetoacetic acid (Ave. 4.81 mgms. per cent), and total urinary acetone bodies (Ave. 13.24 mgms. per cent) calculated as acetone.

Fifty-nine blood analyses, calculated as acetone, on heifer calves from birth to seven months of age averaged 0.70 mgms. per cent acetone and acetoacetic acid, and 1.30 mgms. per cent total blood acetone bodies. An average of 55 urine determinations on this group was 2.54 mgms. per cent acetone and acetoacetic acid, and 4.86 mgms. per cent total urinary acetone bodies. Seven analyses immediately after the completion of parturition averaged 2.54 mgms. per cent total blood acetone bodies for the dam and 1.43 mgms. per cent for the calf.

P10. Glucose Therapy in Ketosis in Cattle. J. C. SHAW AND ROSS C. POWELL, JR., University of Connecticut.

The intravenous injection of glucose in most cases of severe ketosis usually produces a small decrease in blood and urinary acetone bodies for a few hours followed by a rise in acetone bodies often back to the previous level. Pumping from 6 to 10 pounds of glucose into the rumen by means of a stomach tube results in a much greater rise in blood glucose over a longer period of time and causes a marked fall in blood and urinary acetone bodies. In several cases the blood acetone bodies in severe ketosis decreased from a level of 30 to 60 mg. per cent to 3 to 4 mg. per cent within 24 to 48 hours. However, it was found necessary in all cases where the ration was not changed to continue some type of sugar therapy at a rather high level for several weeks. In those cases where the animal had a fairly good appetite it was possible to alternately cure and then reproduce severe ketosis merely by feeding three pounds of glucose per day for a few days followed by removal of glucose from the ration. In most cases of severe ketosis, however, it was found necessary to administer glucose in some other way, usually by

stomach tube, because of the difficulty involved in getting the animals to eat any kind of sugar or molasses.

Blood glucose values in 6 cases of severe ketosis ranged from 15.0 to 30.5 mg. per cent while blood acetone bodies ranged from 23.3 to 70.0 mg. per cent. Blood lactic acid was also low in most cases of severe ketosis ranging from 2.5 to 7.0 mg. per cent. Following the pumping of glucose into the rumen of the ketosis cow there is usually an increase of from four to five fold in the blood lactic acid in from 8 to 30 hours, probably due to the sudden oxidation of large quantities of glucose by the body tissues. The blood lactic acid value at this time often exceeds normal by 200 to 300 per cent.

Data on blood and urinary acetone bodies and blood glucose and lactic acid in ketosis cows and the effect of glucose therapy upon these substances will be presented.

P11. Progress Report on the Relation of the Ration to the Composition of Milk. E. B. Powell, Ralston Purina Company.

In November, 1938 and in June, 1939, progress reports were submitted at the American Society of Animal Production in Chicago and at the Dairy Science meeting at Pullman, Washington.

Early work indicated a correlation between rumination and butterfat test. Rumenless cows might throw some light upon this subject. At the June 1939 Dairy Science meeting, reference was made to the removal of the rumen from several calves. Three of these completely recovered, but as they developed, their rumens were regenerated, with one possible exception.

We again turned our studies to cows in production. To date, we have used 51 cows for 99 lactations. Recently we have confined our studies mostly to the effect of rumination and fermentation on the fat percentage. Twenty-two cows, mostly Holsteins, whose roughage was reduced to 6 pounds of normal alfalfa per head per day with concentrates full fed, showed the usual decrease in fat content of the milk. This decrease persisted as long as this method of feeding was continued, even throughout the entire lactation.

Believing that the contents of the rumen of these cows were abnormal, three cows after showing the typical decrease in fat content, were fed their usual concentrate, wetted and fermented with small quantities of rumen material taken from normal cows at time of slaughter. These cows all responded with an average increase of approximately 1 per cent in fat.

In order to determine if it was necessary for feed to go through a normal rumen before it would be effective, we placed several typical cows on the usual concentrate after it had been fermented for 48 hours. These cows all showed an increase in fat content of the milk thus proving that rumination was not essential.

Method of fermentation: The concentrate was saturated with tap water,

incubated at room temperatures ranging from approximately 100° to 110° Fahrenheit. The average relative humidity in this room was 49 per cent. The material was stirred daily at 4 A.M., 7 A.M., 10:30 A.M. and 4:30 P.M. After 48 hours of fermentation it was full fed to the cows in place of their dry concentrate.

Is fermentation essential? Four cows that had shown the usual decreased fat, received this same concentrate wet, but unfermented. These cow all continued with the low fat tests. This indicated that fermentation was necessary to cause the increased fat content.

The feeding of dry barley malt, butyric acid, and corn sugar mixed separately with the usual concentrate, was tried. Barley malt failed to show consistent increase in fat, while the other two mixtures showed no response at all. Although we have eliminated some factors and are narrowing the problem, we still do not have the solution.

What factor or factors are produced in a normal functioning rumen, or by fermentation? When we know the answers, we believe we will discover one or more of the at present unknown essentials for the production of normal milk and for normal reproduction.

P12. The Influence of Frequency of Milking on Milk Production. L. M. Ludwick, Arless Spielman and W. E. Petersen, University of Minnesota.

As a means of evaluating the influence of frequency of milking on total milk yield, production of the right and left halves of the udder were recorded separately. Thus one half can serve as a check on the other.

Five mature Guernsey cows were used in the experiment. The experimental milkings covered a period of five months. In order to establish a basis for comparing the production of the two halves, preliminary milkings of each half were conducted which covered a period of ten days. The mean production for each half was established and then the frequency of milking was increased from two to three times a day, alternating halves at two-week intervals.

By comparing the records of the halves for the respective frequencies of milking, it was found that three-time-a-day milking had considerable advantage over two-time-a-day milking. Although none of the cows were in heavy lactation, the increase due to one additional milking per day was as high as 16 per cent.

It was also observed that the first half which was milked three times a day seemed to continue at a relatively higher level of production even after twice-a-day procedure was resumed.

This proposed method of using one half of the udder as a check on the other may be of value in experiments regarding milk production where it is desirable to eliminate environmental factors and individual differences.

P13. The Chlorine Tolerance of Certain Mastitis Bacteria. R. K. WAUGH, P. R. ELLIKER, J. H. HILTON AND J. F. BULLARD, Purdue Agricultural Experiment Station.

An attempt has been made to control mastitis in one of the University dairy herds by a sanitary procedure involving the use of chlorine solutions. The procedure consisted of washing the cows' udders before milking with individual cloths saturated with a solution containing 400 p.p.m. available chlorine, rinsing the teat cups of the milking machines between cows in a solution containing 200 p.p.m. available chlorine, and dipping the teats of the cows in a solution of 400 p.p.m. available chlorine after milking.

Experiments were carried out in the laboratory to determine whether or not the chlorine tolerance of certain mastitis organisms was low enough to warrant such a procedure. Also, the efficiency of the chlorine solutions in removing mastitis organisms from the teats was determined. The stability of chlorine solutions in actual use in the barn likewise was determined.

The chlorine tolerance of the respective organisms was determined by exposing standard suspensions to known concentrations of chlorine. Because of the addition of small amounts of milk to the chlorine solutions in actual use in the barn, some trials were run with chlorine solutions containing one per cent skim milk. When milk was added, the concentration of available chlorine was determined after adding the milk and the chlorine solution was then used immediately. One cc. quantities of the standard suspensions of the respective organisms were placed in 99 cc. of the respective chlorine solutions, and, after 20 seconds exposure to the chlorine, one cc. quantities were transferred to sterile N/10 sodium thiosulfate dilution blanks. Then plate counts were made and percentage survivals determined. The source of chlorine was a commercial calcium hypochlorite.

It was found that strain 090 of *Streptococcus agalactiae* would not survive an exposure to five p.p.m. available chlorine for 20 seconds. When one per cent skim milk was added to the chlorine solution, the tolerance of the organism was between 30 and 40 p.p.m. available chlorine for 20 seconds. A similar chlorine tolerance was shown by an alpha type hemolytic streptococcus isolated from a chronic case of mastitis. A strain of *Staphylococcus aureus* isolated from an acute case of mastitis showed considerable resistance to chlorine. With one per cent skim milk in the chlorine solution, 2.3 per cent of the staphylococci survived 600 p.p.m. available chlorine and 16.3 per cent survived 475 p.p.m. available chlorine for a period of 20 seconds.

Where no skim milk was present in the chlorine solution, the staphylococcus was killed by 100 p.p.m., but not by 50 p.p.m. of available chlorine during a 20 second period of exposure. Attempts were made by means of chlorine solutions to kill mastitis streptococci on the teats of cows after inoculation of the surface of the teats with suspensions of the organisms. Despite the low chlorine tolerance of the mastitis streptococci, these organisms could

be recovered from the teats after dipping in a solution containing one per cent skim milk and 400 p.p.m. available chlorine. However, fewer organisms were recovered and the recovered organisms showed less activity when 400 p.p.m. available chlorine rather than 200 p.p.m. available chlorine were used. When the same procedure was carried out with *Staphylococcus aureus,* the chlorine solutions appeared to have little effect upon these organisms.

A solution of calcium hypochlorite was used for rinsing the teat cups of the milking machine between cows, and it was found that after milking 20 cows, the solution contained about 55 per cent of its original concentration of available chlorine. After dipping the teats of 20 cows, the chlorine solutions used contained about 90 per cent of their original concentration of available chlorine.

P14. Influence of Oat Juice Extract upon the Age of Sexual Maturity in Rats. E. T. Gomez, A. M. Hartman and L. P. Dryden, Bureau of Dairy Industry, United States Department of Agriculture.

It has been reported by us (Proc. Am. Soc. Biol. Chem., 1941 meeting) that the juice of young oat plants contains a material which when fed to rats from weaning (21–22 days of age) produces early vaginal opening and stimulates early ovarian activity. The material is precipitated from the oat juice with alcohol. This precipitate has been found active when fed at 2 to 10 per cent of the ration. The average age of vaginal opening of 37 rats fed this precipitate is 29.5 days and 37 litter mate controls not fed the precipitate 45.3 days. Sixteen of 17 experimental rats autopsied showed evidence of stimulation of ovarian activity. The activity of these extracts was not due to the ash in them.

In the above work it was noted that the rats fed ad libitum on the experimental ration generally consumed up to the time of vaginal opening more feed than litter mates on the basal ration during the same time. Paired feeding trials have now been conducted to determine whether or not the effects of the oat juice precipitate noted above, are due to the increased food consumption.

Five pairs of rats were used in this experiment. The oat juice precipitate was fed at 4 per cent of the ration. The feed consumption of the animals fed the oat juice precipitate was in each case a little lower, up to the time of its vaginal opening, than was the consumption of their paired litter mate controls during the same time (0.5 to 4.2 grams less for the period of feeding), but in every case the vagina of the animal fed the oat juice preparation opened before that of its paired litter mate, in fact the vagina of every animal receiving the experimental ration opened before any of the controls. There, therefore, appears to be a specific material in the juice of

the young oat plants that stimulates vaginal opening and ovarian activity. A similar material also appears to be present in milk.

P15. The Effect of a High and a Low Protein Ration on the Gonadotropic Content of Male Rat Pituitaries.* E. J. WEATHERBY AND R. P. REECE, New Jersey Agricultural Experiment Station.

An assay method was developed whereby it was possible to estimate the gonadotropic content of a single male rat pituitary gland by observing its influence on the ovaries of sexually immature rats.

Twenty-two mature male rats were paired according to body weight and age. A 30 per cent protein ration was fed to one member of each pair while a 15 per cent protein ration was fed to the other member. The feeding period lasted from 28 to 62 days. Paired rats were always sacrificed on the same day, their pituitary glands removed and assayed for their gonadotropic content.

Pituitary glands from rats fed a high protein ration contained slightly more gonadrotropic hormone than did the glands from rats fed a low protein ration. The difference, however, was not statistically significant.

P16. The Evaluation of Fertility in Dairy Bull Semen.† H. A. HERMAN AND ERIC SWANSON, University of Missouri.

In this investigation the physical and chemical characteristics of 342 semen ejaculates, secured from 51 dairy sires by means of the artificial vagina, have been studied and the findings correlated with the actual breeding record of the bull. Characteristics studied included volume, initial motility, pH, survival time in storage (both diluted and undiluted) at 40° F.; spermatozoa per cu. mm., and morphological abnormalities of the sperm.

The semen was found to vary widely in all properties studied, though variations in initial motility and pH were of somewhat lesser nature. The greatest variations occurred in the length of time vigorous motility persisted and in percentage of abnormal spermatozoa.

Morphological abnormalities of the sperm were found in every sample of semen studied and ranged from 2 to 74 per cent of the total spermatozoa. Bulls which produced semen averaging over 30 per cent abnormal spermatozoa were usually of poor fertility, but not all samples of semen containing 30 per cent abnormal sperms were infertile. The most frequent types of abnormalities of the spermatozoa found were coiled tails, tailless, and pyriform. Usually the bulls showing a preponderance of coiled tail spermatozoa were of low fertility.

* Journal Series Paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Dairy Husbandry.

† Contribution from the Department of Dairy Husbandry, University of Missouri Agricultural Experiment Station Journal Series No. 738.

Semen of high pH, 7.00 or higher, was usually of low viability. Semen poor in initial motility likewise survived only a short time after collection. As a rule normal pH, strong initial motility, and a low percentage of abnormal spermatozoa were an indication, but not assurance of good fertility.

Fertility of good quality semen was maintained 3 to 6 days when stored at 40° F. The ratio of services per conception for each bull has been determined and the summarized results will be presented.

It is believed that the fertility of a bull cannot be accurately estimated from a single semen sample using the criterions now in common use. Three or more semen samples examined several days, or even weeks apart, with accompanying records of the bull's actual breeding record, provides the most accurate method for evaluating fertility.

P17. The Effect of Exercise on the Amount and Quality of a Dairy Bull's Semen. O. L. LEPARD, C. EDMUND SHUART AND ARDEN FOSTER, New Jersey Agricultural Experiment Station.

With the introduction of artificial insemination on a unit basis, it became necessary to produce semen of the highest possible quality. Exercise is known to aid in keeping bulls in a healthy and vigorous condition. It was the purpose of this investigation to determine the immediate effect of exercise on the amount and quality of semen.

Eight bulls, varying from two to five years of age, were divided into two nearly equal groups according to their age, weight and previous breeding records. All animals were fed and managed similarly and in a normal way except for exercise.

During the first four weeks, all bulls were tied in stalls. At the end of this four weeks period, and for the remainder of the sixteen weeks' experiment, four of the bulls were put on a mechanical exerciser for forty minutes each day. The other four bulls were left tied in the stalls.

Semen (two ejaculates) was collected by means of a standard artificial vagina. These samples were examined for concentration, and smears were made for morphological studies. The semen was then mixed with egg yolk dilutor and used for artificial insemination where conception rate was followed. A portion was stored at 40° F. and motility was read daily at 100° F. until no motility was noted.

The volume of semen showed a slight but insignificant advantage to bulls receiving exercise. No significant differences were noted between the two groups in the factors of morphology, concentration of the sperm, and life of the sperm at 40° F. Conception rates of the two groups of bulls followed the same general trend. These results must not be interpreted as showing that exercise of bulls is of no value. They only show the value of exercise on the amount and quality of semen as determined by the well measurements for a sixteen weeks' trial.

P18. Some Factors Influencing the Reproductive Efficiency of Louisiana Herds. D. M. SEATH AND C. H. STAPLES, Louisiana State University.

An examination of the eleven-year reproductive history of the North Louisiana Experimental (Bangs-free) Herd revealed a relatively low breeding efficiency and stimulated interest in studying the breeding records of the Louisiana State University Herd located in south Louisiana.

When measured by services per conception, the Experimental Herd averaged 2.8 while the University Herd had the creditable lifetime average of 1.7 for 934 cows leaving the herd between 1923 and 1940. In spite of the difference in rate of conception, each herd had almost identical calving intervals, averaging between 13.5 and 14.0 months.

Seasonal differences in rate of conception were found for both herds. In each case the summer months required the most services per conception. The best record for rate of conception in the Experimental Herd was during the winter months and the fall months were second. The University Herd had a reverse in this order with the fall months first and the winter months second.

About one-half (18) of the first-gestation heifers in the experimental herd had an extremely low rate of conception, averaging 5.3 services per conception, while the others (22) average only 1.4 services per conception. A similar division took place for the second-gestation group. These two groups of young cows with low-breeding efficiency contributed much toward the poor reproductive history of the herd.

Within the University Herd 65.7 per cent of the conceptions resulted from a single service, 18.2 per cent from two, 8.4 per cent from three, 4.1 per cent from four, and 3.6 per cent from five or more. As the number of services increased from one to five, the calving interval increased by six months.

Differences in breeding efficiency were found as between years, sires and dams within each of the herds. Natural soil fertility and annual rainfall favored the production of pasture and cultivated crops in the southern regions of the state thus suggesting a possible explanation for the differences found in the breeding efficiency of the two herds studied. The fact that each herd had the lowest breeding efficiency during the summer months suggests the influence of factors related to climatic changes.

P19. Progress Report on a Roughage Program in Herd Management. C. B. BENDER, New Jersey Agricultural Experiment Station.

High quality roughage is considered an ideal feed for dairy cattle. The quality, however, is dependent on many factors such as soil, fertilization, climate, plant source and the manner in which the roughage is preserved.

This study, covering a period of five years, involves a program of feeding

large amounts of grass silage of various kinds, pasture, and limited hay feeding, on the growth and productive ability of Holstein and Guernsey cattle. One hundred and fifty-nine milking animals and 216 heifers were included in the study.

Holstein milking cows were fed for the most part from 50 to 70 pounds of grass silage a day and the Guernseys received between 40 and 50 pounds depending on their size and ability to handle it. Hay consumption was limited to an intake of 6 to 10 pounds a day in addition. Grain was fed in proportion to production, the Guernseys being fed at the ratio of 1 : 3.5 while the Holsteins averaged 1 : 4 in the winter. During the pasture season, the bulk of the roughage was furnished by the pasture in the flush period. When pastures were short, grass silage or hay was supplemented.

Heifers at the age of 12 to 15 months were placed on an all roughage diet of either pasture, grass silage or a combination of grass silage and three pounds of hay. They continued on this diet until two months before freshening when they were finished off on grain.

All production records were made on twice-a-day milking; the lactations were divided into 305 days or over, and under 305 days. The milk production was calculated on a 4 per cent fat basis. For the growth data, the animals were weighed and measured every three months after they reached the age of 12 months.

This study shows that (1) normal production of milk seems to be maintained on this method of feeding; (2) the growth curves are slightly under Ragsdale standards for height and heart girth. Weight normality drops as low as 85 per cent when the animals are 24 months of age and rises to 98 per cent when the animals are 3 years old. The heifers were fed timothy silage for the first three years and oats and peas silage for the last two years in the growth studies. Legume silage or a mixture of legumes and grasses was fed to the cows in production.

P20. The Approved Ayrshire Sire Program. C. T. Conklin, Ayrshire Breeders' Assn.

The Approved Ayrshire sire plan, based on the results of some ten years of personal study on the part of Leonard Tufts of Pinehurst, North Carolina, was adopted on November 15, 1940 by the directors of the Ayrshire Breeders' Association, and is now a service furnished to Ayrshire owners.

The first step in the study of a sire is to list in the order of birth all registered daughters three years old or over, regardless of whether or not they have been tested. Every effort is made to make certain that every daughter of a sire that has freshened in a herd that is on test is included in this study. Sires are not approved when the data show that the tested daughters are a selected group.

All records are computed to a mature equivalent, 305-day lactation basis

on two milkings daily by the use of standard factors approved by the Bureau of Dairy Industry, United States Department of Agriculture. An Ayrshire sire is considered approved when he meets the following requirements, with all records computed to a mature equivalent, 305-day lactation basis:

1. A comparison of a complete sample of at least ten daughter-dam pairs, but including all daughters from tested dams.
2. All tested daughters must average at least 8500 lbs. milk or 340 lbs. fat with an average butterfat test of not less than 3.9 per cent.
3. Sire must have an equal parent index of not less than 8500 lbs. milk or 340 lbs. fat and a butterfat test index of not less than 3.9 per cent.
4. Not less than 70 per cent of all the tested daughters must each make 8500 lbs. milk or 340 lbs. fat.

Although this method of analyzing the transmitting ability of dairy sires may seem rather complex, it is believed that each of the above requirements is essential.

P21. Some Chemical Determinations Useful in Silage Studies. A. E. PERKINS, Ohio Agricultural Experiment Station.

Among the changes which occur in green plant material in the process of silage making probably the most obvious is the disappearance of sugars and the production of acids. This, however, is only one of the many chemical changes which occur. The nature and extent of these changes largely determine the quality of the resulting silage.

Aside from the conventional cattle feed analyses, which certainly do not supply adequate information, the worker is largely "on his own" since there seem to be no well developed or standardized procedures, for tracing the progress and nature of chemical changes which occur during silage making. There is presented here a description of certain determinations which have been found useful in this connection. It is well realized that the field has not been fully covered and that perfection has not been attained in any case.

Dry Matter. This determination is always included since we regard dry matter control as probably the most important single factor in determining silage quality. A rapid and reliable method for making this determination has been found and described elsewhere.

Nitrogen Separations. The proteins and related nitrogenous compounds as well as the sugars undergo marked changes during silage making. The series of determinations outlined here gives a fair idea of the extent and character of these changes.

The samples examined in the cases of both the crop and the silage include:

a. The entire material.
b. The expressed juice or a 4:1 water extract in the case of samples too dry to yield juice.

c. The filtrate from (b) after adjusting acidity and heating to coagulation.

Total Nitrogen determinations are conducted on (a), (b), and (c). Ammonia determinations (aspiration technique) are conducted on (b) and (c). The ammonia determination in silage is of especial significance since it seems to correlate closely with the quality of the silage. Amino Acid determinations are conducted by the Sorenson Formal Titration method on (b).

Titration Curve. Replacing and amplifying the determination of pH and titratable acidity, titration curves are now made on (b) which are thought to provide additional valuable information.

Acids. A disagreeable, penetrating, and persistent odor found in some silages particularly that made from very wet material is frequently attributed to butyric acid. The exact extent to which butyric acid replaces acetic acid in the volatile acid fraction of the silage acids in such cases has not been thoroughly studied. Methods heretofore used for the separate determination of the individual acids occurring in silage are cumbersome and unsatisfactory.

Studies are under way and some progress has been made toward simplifying the methods.

P22. Corn Meal as a Grass Silage Preservative. G. Bohstedt, W. H. Peterson, and G. P. Bahler, University of Wisconsin.

More than 20 years ago in several experiments ground corn grain was used with fair success in the preparation of legume silage. Meanwhile corn has become almost forgotten as a grass silage preservative. It has seemed that compared with a more readily fermentable carbohydrate like sugar or molasses, or compared with phosphoric acid or the A.I.V. acid mixture, ground corn was a less logical preservative. But recent tests at the Wisconsin Experiment Station have indicated that rather effective preservation may be obtained provided enough ground corn is added. It appears that a larger proportion of the starch of corn grain is converted to preservative acids than has heretofore been thought to be the case. If then corn has at least fair preservative properties, a farmer with corn on hand at the time of grass silage harvest may save out-of-pocket cash that is necessary for buying molasses or acid.

The Wisconsin experiments conducted during the past 3 years have included corn meal-alfalfa silages put up in bottles, barrels, and as 5 to 10 ton layers of silage in tower silos. Samples were analyzed for pH, carotene, and in a number of cases for NH_3–N. Where corn meal-alfalfa silage had been fed for a considerable time to milking cows, the milk was analyzed for carotene and vitamin A. The total vitamin-A potency of the milk was found to be about equal to that of milk from molasses-alfalfa silage.

When less than 120 pounds ground corn was used for a ton of green alfalfa, the quality of the resulting silage as based on appearance, odor, and chemical analysis was only fair. Adding 2 pounds of sulphuric acid to the limited amounts of corn per ton of alfalfa gave much better preservation of carotene.

From 150 to 200 pounds ground corn as a preservative for alfalfa silage gave rather good results in all of the above aspects, and compared in its effect with 60 pounds of molasses. An outstanding effect of ground corn as a preservative was the improved palatability of such alfalfa silage as compared with alfalfa silage produced in various other ways. It is suggested that shelled corn or ear corn which is to be used as a preservative, be ground rather fine.

P23. Trench Silos for Preserving Cereals Treated with Molasses, or Phosphoric Acid.*

H. A. HERMAN, A. C. RAGSDALE AND WARREN HEATHMAN, University of Missouri.

Dairy farmers frequently desire to store cereals, often grown as pasture or cover crops, in temporary silos. Previous experience at this Station and in field trials had demonstrated that the "paper sack" or "snow fence" type of silo was not too well suited for this purpose because of excess spoilage during the summer months. We, therefore, in the summer of 1940, conducted trials using trench silos for barley.

Two trench silos, with a capacity of 45 to 50 tons each, were used. The silos were located on a well drained hillside. The walls of the silos were 12 feet apart at the top and 8 feet at the bottom of the trench. Each silo was filled one-half with chopped and one-half with unchopped barley bundles. The barley was cut in the milk stage.

One of the silos was filled with barley silage treated with 60 pounds of blackstrap molasses per ton. In this silo 32.15 tons of barley was chopped into ½ to 1″ lengths, and stored at one end of the silo. In the opposite end 20.12 tons was stored unchopped. A binder was used for harvesting the green barley and the bundles were put into place in stack fashion. Each layer was treated with blackstrap molasses.

In the second silo 26.04 tons of chopped barley, treated with 8 lbs. of 75 per cent phosphoric acid per ton, was placed in one section. The remaining space was filled with 19.05 tons of bundles and treated with acid at the same rate as the chopped barley.

The silos were sealed and precautions taken to prevent surface water from draining into the pits.

Six months after filling, the silos were opened and the contents fed to cows in milk and also to growing heifers. The spoiled portions, as well as

*Contribution from the Department of Dairy Husbandry, University of Missouri Agricultural Experiment Station Journal Series No. 737.

the good quality silage, was weighed from each silo. The palatability and relish with which the cattle ate the silage was carefully noted. Only good quality silage was offered the animals.

The losses through spoilage in terms of fresh silage were as follows:

1. Chopped barley—molasses treated 16.90% spoilage
2. Barley bundles—molasses treated 26.5 % spoilage
3. Chopped barley—phosphoric acid treated 14.32% spoilage
4. Barley bundles—phosphoric acid treated 15.22% spoilage

With the exception of bundles treated with molasses the rate of preservation for a temporary type of silo was apparently satisfactory. Earlier studies have shown losses of 13 to 28 per cent of the total silage made from corn when using permanent silos. The greater spoilage in the molasses treated bundles was largely due to mold growth and excess fermentation no doubt as a result of inability to secure sufficient packing in this type of silo so as to exclude air. The phosphoric acid silage made from bundles was preserved nearly as well as the chopped barley.

A short feeding trial using the reversal method with 8 cows of equal weight and producing ability indicated no significant difference in the palatability of the two silages, although if any difference the cows ate the molasses silage most rapidly. The groups receiving molasses silage averaged approximately one pound more milk per day, but this difference was not considered significant.

Analyses of the fresh barley have been made, and analyses of the silages are under way in an attempt to measure losses in nutrients.

These trials indicate that trench silos built and filled according to the best practices may be satisfactorily used for ensiling chopped cereals treated with either molasses or phosphoric acid. Where unchopped cereals are ensiled care must be used to insure proper packing to prevent excess spoilage.

P24. Calculating Pasture Yields with Dairy Heifers as Experimental Animals. H. B. MORRISON AND FORDYCE ELY, Kentucky Agricultural Experiment Station.

Data are reported from five years (1936–1940, inc.) experimental grazing of bluegrass pastures on which Jersey and Holstein heifers were used. Half of the pasture was grazed continuously throughout the season and the other half was divided into two equal portions which were pastured alternately for two week periods while the grazing season lasted. Heifers for the experimental pastures were allotted on the basis of age, breed, breeding, weight, height at withers, and nearness to figures for normal weight and height as published by the Missouri Agricultural Experiment Station (Bul. 336, 1934). All heifers were weighed on three consecutive days in alternate weeks and from these weights were computed the gains or losses and the weight of the heifers maintained daily per acre on the pastures throughout the season. The figures obtained for gain or loss are converted into terms

of T. D. N. on the basis of the recommendations of the pasture research committee of the A. D. S. A. (Rev. of Mimeo. Circ. 1046, U. S. D. A., Soil Cons. Serv., June 1940), 3.53 pounds T. D. N. per pound gain in weight and 2.73 pounds T. D. N. per pound loss in weight. The average weight of all (94) of the heifers involved (624 lb.) was used as a guide in calculating the T. D. N. for maintenance. Based on data from "Feeds and Feeding" (Henry & Morrison) approximately 4.65 lb. T. D. N. daily is required to maintain a 624 lb. heifer.

The "Bluegrass equivalent," a term used to give an approximate figure for pasture yield, is calculated from the total T. D. N. for maintenance and gain, using the figure given for T. D. N. for bluegrass (all analyses) in "Feeds and Feeding."

P25. A Study of the Relationship of Fat Content in the Dairy Grain Ration to Milk and Butterfat Production. C. F. MONROE AND W. E. KRAUSS, Ohio Agricultural Experiment Station.*

In a series of four feeding trials two grain mixtures containing different amounts of fat have been compared. These grain mixtures were alike except for the protein supplement used and were composed of such common feeds as corn, oats, wheat bran, beet pulp and molasses. Ground soybeans and expeller soybean oil meal were used in the higher fat mixture, and extracted soybean oil meal in the lower fat mixture. The former mixture contained, approximately, 4.5 per cent fat and the latter 3.0 per cent fat.

In three of the trials to be reported the feeding program has consisted of a preliminary period of 30 days and an experimental period of 100 days. The roughage feeding in these three trials has consisted of fair to good quality clover or alfalfa hay and corn silage. The hay has been fed *ad libitum* and the corn silage at the rate of 30 pounds per day. In a fourth trial, the amount of roughage fed was calculated to satisfy maintenance, and sufficient grain was fed to furnish 80 per cent of the theoretical T.D.N. requirement for milk production. During the preliminary period all cows have received the same grain mixture consisting of a 50–50 blend of the experimental mixtures indicated above.

Of the two trials completed at the time of writing this abstract, one has shown an advantage for the higher fat mixture, whereas in the other the results are practically equal.

The remaining two trials will be completed and the results will be available by the time of the meeting.

P26. The Influence of Sustained High Fat Intake upon Milk Fat Production. N. N. ALLEN AND J. B. FITCH, University of Minnesota.

Large increases in fat intake of dairy cows by addition of any of a num-

* In cooperation with the Ohio State Department of Public Welfare and the Central Soya Company, Inc., Fort Wayne, Indiana.

ber of common fats have been shown to cause an immediate increase in the fat content of the milk and in milk fat yield, but the influence of continued high-fat intake has not been demonstrated.

In 1939 the influence of six different fats, including butterfat, lard and linseed, coconut, soybean and corn oils, fed at a level of 1.5 pounds daily over fifty-day periods was studied in comparison with a ration similar in every respect except that 3.4 pounds of a mixture of equal parts of cornstarch and sucrose replaced the fat. Two cows in early lactation and as nearly matched as possible for productive level were used for each of the fats. The reversal plan was followed with each pair of cows.

The cows receiving butterfat and lard showed decidedly higher test and milk fat production during the period of high-fat intake than during the period of low-fat intake. Slight increases were observed with linseed and coconut oils and marked decreases for soybean and corn oils. A similar trial was carried out in 1940 using butterfat, lard, tallow and coconut, cottonseed, peanut, soybean and corn oils.

The fat content of the milk and the yield of milk fat were markedly higher during the period of high-fat intake with butterfat, lard, tallow and coconut and cottonseed oils, and slightly higher with peanut oil. With soybean oil the test was slightly lower. With corn oil pronounced decreases in both test and fat yield were observed.

With all fats except the soybean and corn oils the results are in agreement with those observed previously with five-day periods. The stimulating influences appeared to be exerted throughout the fifty-day periods, although greatest immediately after the change of rations.

With soybean oil and with corn oil the sustained effect seemed to be in direct contradiction to that previously noted over five-day periods. Comparing the production for the five days immediately preceding and following the change of fat intake, it was found that the test was higher in the high-fat period with soybean oil. With corn oil the test was slightly lower when the oil was fed, but the depressing effect was not as great as that observed with continued feeding. This suggests the presence of a depressing agent in the corn oil and possibly in the soybean oil which is slow in exerting its influence to the point of overcoming the stimulating influence which other constituents of the fat might be expected to have. Further work is being carried out with a greater number of cows to determine whether this depressing action is characteristic of corn oil and to determine the causative factors.

P27. The Relation on Mineral Intake and Sunshine to the Vitamin D Deficiency of Mature Dairy Cattle. G. C. WALLIS, South Dakota Agricultural Experiment Station.

In continuing our studies on the vitamin D deficiency of mature dairy cattle we have now accumulated considerable evidence as to the relation of

the level of calcium and phosphorus intake to the development of vitamin D deficiency symptoms. Two methods of approach have been used, namely: (1) the development of vitamin D deficiency symptoms on a ration supplying a normal mineral intake with subsequent addition of liberal amounts of calcium and phosphorus to note curative effects, if any, and (2) the feeding of vitamin-D-deficient rations supplying two or more times the normal amount of calcium and phosphorus to see if deficiency symptoms would develop. Detailed evidence has been obtained including observations on the physical condition of the animal, the level of calcium and inorganic phosphorus in the blood plasma, the concentration of vitamin D in the blood plasma and butterfat, and on mineral balance trials. The combined evidence indicates that generous amounts of calcium and phosphorus in vitamin D deficient rations will not prevent the development of deficiency symptoms in dairy cows. Neither will the addition of calcium and phosphorus to the ration of animals exhibiting evidence of vitamin D deficiency relieve that condition.

Observations of a similar nature have been made on other cows while deficiency symptoms were developing and after exposing such vitamin D deficient animals to the antirachitic effects of sunshine. Certain cases studied during the late fall and winter are essentially significant in giving direct evidence of the antirachitic effectiveness of sunshine at this season of the year. In this experiment a direct attack on the problem has been made in that the cows were first depleted of vitamin D reserves so that conditions were optimum for noting any possible effects of sunshine exposure. The evidence indicates that mature dairy cows respond favorably to the antirachitic effects of sunshine in a manner similar to that which has already been demonstrated for calves. Also, that the vitamin D potency of the butterfat produced may be increased by this means.

P28. Observations on the Quantitative Requirement of Mature Dairy Cattle for Vitamin D. G. C. Wallis, South Dakota Agric. Experiment Station.

The quantitative requirement of mature dairy cows for vitamin D becomes a question of some significance now that it has been demonstrated that normal health and vigor cannot be maintained without it. Studies under way at this station on the vitamin D deficiency of dairy cows have already given some information on this problem. Five different cows showing various degrees of vitamin D deficiency have been given known amounts of vitamin D usually in the form of a limited quantity of alfalfa hay of known vitamin D content. The results have been measured in terms of the amount of vitamin D required to initiate healing and the rapidity and degree of the resulting recovery. Observations on the physical condition of the animal, changes in the concentration of blood plasma calcium and inorganic

phosphorus, the vitamin D in the blood plasma and butterfat, and mineral balance trials have contributed to the interpretation of the results.

An idea of the magnitude of the requirement is indicated by the fact that in one case 2 pounds of alfalfa hay sufficed to bring about marked improvement in the physical condition of a vitamin D deficient cow and bring the blood plasma calcium and inorganic phosphorus from very low levels to normal concentrations in about four weeks' time. The available data is now being tabulated for further study so that more definite conclusions will be available in the near future.

P29. Relation of Skeletal Reserves of Calcium and Phosphorus Laid Down During Growth to Persistence of Milk Production of Dairy Cows. L. S. Palmer and T. W. Gullickson, University of Minnesota.

Two groups of seven pure-bred calves, each group represented by three Guernseys, two Holsteins and two Jerseys, were reared from weaning on rations which differed only in their calcium and phosphorus content and were continued through the first two lactation periods on rations which differed in a similar manner. The control group received liberal amounts of calcium and phosphorus; for three animals these averaged about 70 grams calcium and 30 grams phosphorus daily per 1000 pounds weight; for the remainder these averages were approximately 125 grams calcium and 35 grams phosphorus. For each control animal there was a corresponding experimental animal of the same breed which differed from it only in receiving in its grain mix enough steamed bone meal to double the phosphorus intake. After calving the calcium and phosphorus intakes of the control animals were determined largely by the grain intake commensurate with the milk production but the bone meal group received daily 100 grams of bone meal plus an additional 20 grams per pound grain fed. With one exception all animals were on official test for the first lactation and on herd ration for the second lactation; one animal in the bone meal group was on official test for both lactations. In general the bone meal group consumed approximately 150 grams bone meal daily throughout the entire experiment.

Two grade calves, one in each group, were reared to the age of 28 months but not bred. Bone composition studies showed heavy deposits of calcium and phosphorus which were slightly greater for the bone meal fed animal. Bone analyses of one of the latter group of pure-breds after her second lactation showed that the heavy deposits of calcium and phosphorus had not been impaired.

A study of blood plasma calcium and inorganic phosphorus at monthly intervals throughout the entire study showed that the phosphate concentration was definitely higher up to the first parturition for the bone meal fed heifers paired with the controls receiving the lower level of calcium and

phosphorus but this difference was not so striking, and in some instances not evident, in the other pairs. After lactation started the differences were not marked for any of the pairs.

There was no appreciable difference in rate of growth of the animals in the two groups but the bone meal fed animals seemed to have a somewhat better general appearance during this period. There were no differences in breeding capacity or in the incidence of difficulties attributable to the feeding or lack of bone meal. Actually eight animals were started in each group of pure-breds but one Holstein in each group could not be considered in the final results because of accidents not related to the experiment.

The effects of the bone meal feeding on persistence of milk production were judged on the fat-corrected milk basis both for 365 day (or less) lactations and on the complete lactation periods. At first sight the results seemed significant because the mean differences between the first and second lactations of the two groups were 1066 pounds for the 365 day lactations and 1642 pounds for the complete lactations, both differences being in favor of the bone meal fed group. However, the statistical P-values of these differences were only 0.28 and 0.32, respectively, indicating that they were not significant.

P30. The Effect of Feeding Chloretone (Trichlorobutyl alcohol) on the Blood Plasma Ascorbic Acid of Dairy Cattle. A. L. BORTREE, E. C. SCHEIDENHELM, AND C. F. HUFFMAN, Michigan State College.

Chloretone which had been observed to increase ascorbic acid excretion in the urine of rats was fed at different levels for varied lengths of time to dairy cows and bulls and the effect on the plasma ascorbic acid observed.

Six cows which were open or in the early stages of pregnancy and one cow in the fifth month of gestation showed ascorbic acid levels within the normal range prior to the period of feeding chloretone. One open cow and two bulls were below this level.

One cow given a 40 gram dose of trichlorobutyl alcohol showed an increase in the plasma ascorbic acid the following day and a peak was reached on the seventh day. One open cow fed 10 grams daily showed an increase after five days of feeding and reached a peak on the ninth day; a cow in the fifth month of gestation did not respond to feeding chloretone at this level.

When three cows were fed at the five gram level the response was noted on the third day in two of the cows and on the fourth day in the third cow. The peak was reached on the same day that the response was noted in these three animals.

The levels of plasma ascorbic acid were appreciably increased in the two bulls which were fed 5 grams of chloretone per day.

When 5 grams of chloretone were fed per day the plasma ascorbic acid level increased about 50 per cent; when larger doses were given an increase

of approximately 100 per cent was observed. The ascorbic acid values returned to normal in about two weeks after the trichlorobutyl alcohol feeding was discontinued.

Five grams of chloretone per day for a period of 30 days had no noticeably bad effects on the animals but higher levels of feeding had an anesthetic effect accompanied by lowered milk production.

P31. Some Observations on the Carotene Content of the Blood Plasma of Dairy Cows.

HAROLD GOSS AND S. W. MEAD, University of California.

In connection with studies on the relation of carotene to rancid flavor in milk, carotene values were determined on blood plasma of cows, (five Jerseys and one Guernsey) taken from pasture and placed on a low carotene diet consisting of bleached alfalfa hay, testing less than 0.05 mgm. of carotene per 100 grams, and concentrates of negligible carotene content. When the plasma carotene had fallen to a minimum level the cows received (1) fresh green alfalfa of known carotene content; (2) crystalline carotene dissolved in cottonseed oil, orally; (3) intrajugular injections of crystalline carotene suspended in blood serum; and (4) intrajugular injections of crystalline carotene dissolved in cottonseed oil.

The depletion of carotene in the blood of all of the cows while receiving bleached hay followed the same type of curve, with a very rapid fall during the first ten days. Although original blood plasma values varied from 0.90 to 2.30 mgm. per 100 cc. plasma these cows reached a constant value of 0.04 to 0.09 mgm. per 100 cc. blood plasma after 70 to 90 days respectively.

Oral administration of 250 mgm. daily of crystalline carotene in cottonseed oil caused only slight increases in the blood carotene level. Five hundred mgm. daily increased the level to 0.76 mgm. per cent from initial values of 0.05 to 0.08 mgm. per cent in a total of 41 days. Little increase beyond this value was noted with this level of carotene feeding, but when the intake was raised to 1000 mgm. daily for one cow the blood carotene level increased from 0.75 to 2.33 mgm. per cent in 54 days.

Two cows fed 24 pounds daily of fresh green alfalfa with an average value of 33.6 mgm. per pound as fed, or a daily intake of approximately 800 mgm. of carotene showed blood carotene values intermediate between two cows receiving respectively, 500 and 1000 mgm. daily of crystalline carotene in cottonseed oil.

Intrajugular injections of suspensions of from 400 to 900 mgm. of crystalline carotene in blood serum caused no detectable change in blood carotene in a cow whose blood carotene had been depleted to 0.04 mgm. per cent, as indicated by analyses of blood samples taken from the opposite jugular within two and one half minutes following injection and at 15 to 20 minute intervals throughout the day.

With a second cow an intrajugular injection of 250 cc. of cottonseed oil containing 1500 mgm. of crystalline carotene also failed to bring about a significant increase in blood carotene. Four days later a second similar injection of 1100 mgm. of carotene brought about no detectable increase in blood carotene. Analyses of the lungs, liver and body fat made four hours following the last injection of 1100 mgm. of carotene revealed large quantities of carotene in the lungs, relatively small quantities in the body fat and only a slight amount in the liver. Based on these analyses, the lungs alone had retained 1200 mgm. of carotene.

P32. Vitamin A Levels in the Blood Plasma of Dairy Cattle on Winter Rations and the Influence of Vitamin A Supplementation on Certain Constituents of the Blood. PAUL H. PHILLIPS, P. D. BOYER, H. A. LARDY, AND N. S. LUNDQUIST, University of Wisconsin.

The vitamin A, carotene, and ascorbic acid content of blood plasma was obtained from certain herds in three breeding cooperatives. Samples were taken in late October, January, and March or April. The cattle (both bulls and cows) were divided into controls and those receiving a high potency vitamin A shark liver oil for either 2 months or 5 months. Preliminary results indicate a sparing action on carotene, a tendency to maintain more nearly normal ascorbic acid values, and in the case of prolonged feeding to maintain an adequate blood plasma vitamin A.

P33. The Blood Plasma Vitamin A Content of the New Born Calf and Its Relation to Certain Calfhood Diseases. PAUL H. PHILLIPS, NORMAN S. LUNDQUIST, AND PAUL D. BOYER, University of Wisconsin.

The blood plasma vitamin A, carotene, and vitamin C analyses were made on the new born calf at birth and at varying intervals of 12, 24, 48 hours and several weeks after birth. Without exception the ascorbic acid was higher than average and in no case could vitamin A be found in measurable amounts before suckling. The ingestion of colostrum quickly brought the vitamin A and C levels to normal. The relationship of vitamin A to scours and allied ills of the calf will be discussed from data obtained from field experiments.

P34. The Carotene (Provitamin A) Requirements of Dairy Cattle for Lactation. A. H. KUHLMAN AND W. D. GALLUP, Oklahoma A. and M. College.

Prairie hay has been used as the sole source of carotene in a study to determine the vitamin A requirements of dairy cattle for reproduction and lactation. Grade Jerseys have been fed prairie hay at levels which repre-

sent approximately a full, a fifty per cent and a twenty-five per cent normal hay allowance. Eighteen cows have completed thirty-four gestation periods.

When the average daily intake of carotene during the last ninety days before calving was forty or less micrograms per pound of body weight reproduction was quite likely to be impaired. Many of the calves born were very weak, and most of the cows developed abnormal conditions at or soon after the time of calving.

When prairie hay was the only source of carotene an average daily intake of from 40 to 45 micrograms of carotene per pound of body weight was about the minimum amount which met the requirements of Jersey cows for normal reproduction and the initiation of a normal lactation performance.

Preliminary results of twenty-two complete lactations of twelve grade Jersey cows indicate that the requirements of carotene for the function of lactation apparently do not exceed the requirements for normal reproduction. The lowest average daily intake of carotene for the entire lactation was 39 micrograms per pound body weight and the largest 239 micrograms. Carotene intakes during the gestation and lactation periods in excess of the requirements for normal calving did not increase milk and butterfat production of these grade Jersey cows, the butterfat yields of which did not exceed 400 pounds in 310-day lactation periods. Production was greatly reduced when a low carotene intake during the last three to five months of gestation was followed by difficulties at the time of parturition or impairment of vigor during the early part of the lactation period.

There are indications that if a lactation has proceeded in a normal manner for several months on a low but adequate carotene intake, the carotene intake may be reduced considerably lower than 40 micrograms daily per pound body weight for several months without apparently influencing yield adversely. This may mean that the carotene requirements are actually lower for lactation than for reproduction and also, that a higher carotene intake is required at the beginning of the lactation period than in the later months of the milking period.

P35. Further Studies of the Effects of Vitamin A Deficiency on Reproduction.

S. L. HANSARD* AND T. S. SUTTON, Ohio State University, Ohio Agricultural Experiment Station.

Previous work has demonstrated that in male animals (rats and calves) suffering from avitaminosis-A, there is marked degeneration of the germinal epithelium of the seminiferous tubules and reciprocal pituitary changes. It was considered advisable to study further the relative extent of these changes when graded amounts of vitamin A were used to supplement a diet devoid of this factor.

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Three groups of weanling male albino rats were placed on the U.S.P. vitamin A deficient ration. This ration was supplemented with graded dosages of vitamin A from Reference Standard Oil. The amount admintered was maintained at levels of 10, 20 and 30 International Units per kilogram of body weight. After approximately 66 days on this ration the experiment was terminated.

A histological examination of the testes revealed a close relationship between the degree of degeneration and the level of vitamin A intake. By this examination one could determine with striking accuracy the level of vitamin A intake of the animal from which the specimen was prepared. Some evidence of degeneration was noted in the testes of animals receiving 30 I.U. of Vitamin A per kg. of body weight.

An assay of the anterior pituitaries for gonadotropic activity showed interesting differences between groups. The glands from the animals receiving 10 I.U. per kg. daily had the greatest gonadotropic activity per unit weight of pituitary tissue and those receiving 30 I.U. the least, with those on the 20 I. U. level, intermediate in this respect.

At the termination of the experiment the animals were killed by exsanguination and the blood collected for plasma ascorbic acid determinations. This was carried out by the 2–6 dichlorophenol indolphenol titration method. The animals on the 30 I.U. level had an average blood plasma ascorbic acid content of 0.896 mg. per cent, those receiving 20 I.U. per kilogram 0.737 mg. per cent and those receiving 10 I.U. per kg. had an average level of 0.587 mg. per cent.

The data obtained indicates that 30 I.U. of vitamin A per kilogram of body weight is not sufficient to protect the rat from degenerative testicular changes over a long period of time.

P36. Some Ocular Changes and Deficiency Manifestations in Mature Cows Fed a Ration Deficient in Vitamin A. L. A. MOORE, Michigan State College.

Mature cows, fed a vitamin A deficient ration previously used with calves, failed to develop blindness due to constriction of the optic nerve as has been observed in calves. Papilledema was more difficult to develop in cows than in calves and failed to become evident in two out of six animals. This difference is probably explained by differences in intraocular tension of the two age groups. Once the papilledema develops it takes considerable time for it to recede.

Mature cows did develop nyctalopia, incoordination, and an edema of the legs on the vitamin A deficient ration. The tapetum nigrum and lucidum of the eye developed a mottled appearance.

When the plasma carotene values receded to a 0.2 to 0.5 microgram level deficiency symptoms usually followed in a short period of time. The fat

of a Guernsey cow which died with symptoms of vitamin A deficiency showed the presence of a pigment which was most likely carotene since it was epiphoric between petroleum ether and 92 per cent methyl alcohol.

MANUFACTURING SECTION

M1. The Role of Acid Cleaning Agents in Dairy Detergency. M. E. Parker, Beatrice Creamery Company, Chicago, Illinois.

The alkaline cleaning compounds have played an important role in dairy sanitation. Due to their inherent chemical properties, they have long been recognized as best adapted for practically all dairy cleaning practices.

Acid compounds have suffered by comparison because of their inferior detergency and particularly because of their corrosive action upon dairy metals. Recent development of an acidified steam rinse in the cleaning of milk cans indicated the desirability of acid type of cleaners due to the improved bacterial flora surviving such treatment. The use of acid cleaning agents possessing even enhanced detergency, the lack of any appreciable corrosion, the means for preventing as well as reducing milk stone deposits are all important and probably point to a revision—and incidentally a marked improvement—of dairy cleaning practices.

M2. The Value of Acidifying Milk and Cream Cans from the Standpoint of the Effect Upon Quality. Alvin Rippen and L. H. Burgwald, Ohio State University.

Experiments were conducted to note the effect of an acid reaction in milk and cream cans upon the bacterial flora. Acidification was accomplished by means of an acid ejector placed on the last steam jet of the can washer. One hundred cans acidified with about 0.63 per cent gluconic acid had a lower total bacteria count on whey and standard tryptone glucose skim milk extract agar than similar untreated cans. When 100 cc. of sterile water was added to the freshly washed cans and allowed to stand at room temperature for 24 hours, a pH of 5.0 or less markedly inhibited the development of proteolytic bacteria. Greatest numbers of proteolytic bacteria developed in cans having a pH near the neutral point. Little difference was noted in the total bacteria count after incubation. After two days at room temperature, the acidified cans had a better odor than the non-acidified cans.

Acidifying cream cans before returning to direct shippers did not show any significant improvement in the quality of the raw cream received. The titratable acidity of the cream was about 0.45 per cent. Little difference was noted in flavor and total bacteria count; however, cream received in acidified cans had a slightly higher yeast count. Proteolytic bacteria were often absent or present only in very small numbers in high acid cream.

Control samples of raw cream held at 40 to 45° F. and at 55 to 60° F.

showed no significant difference in the number of proteolytic bacteria and total bacteria count, irrespective of container reaction.

Sterile glass containers rinsed with distilled water were used to collect the milk which was added to treated and untreated cans. No noticeable effect of holding raw and pasteurized milk for 72 hours in acidified and non-acidified cans, either as to number of proteolytic or total bacteria, was observed.

Acidifying the cans with gluconic acid as done in these experiments had no detrimental effect upon the can.

Investigators have found butter samples to increase in phosphatase value during storage. Studies were made on creams held in both acidified and non-acidified cans and the butter made from them with respect to their phosphatase reaction. Cream was held in acid and non-acidified cans for one week at 45° F. and at 55 to 60° F., then pasteurized at 145° F. for 30 minutes. Samples of the creams held at 45° F. and at 55 to 60° F. from both types of containers showed no appreciable difference in the time required for the change in phosphatase activity. Samples of the resulting butter gave similar results. A decrease in the number of proteolytic bacteria in cream increases the time necessary to change the resulting butter from a negative to positive phosphatase reaction.

Bacillus subtilis, Achromobacter putrefaciens, Pseudomonas mephitica, and a proteolytic rod isolated from butter produced a positive phosphatase reaction in sterile milk. *Aerobacter aerogenes* was only slightly effective in causing a change in phosphatase results. No change occurred in sterile milk inoculated with *Escherichia coli, Chromobacter viscosum, Proteus vulgaris, Streptococcus liquefaciens, Lactobacillus bulgaricus, Lactobacillus acidophilus, Pseudomonas fragi,* and several others isolated from butter (when using Gibbs reagent).

M3. The Bacteriological Spoilage of Milk Held Near the Freezing Point.

J. M. Sherman, G. M. Cameron and J. C. White. Cornell University.

Inasmuch as our information on the growth of bacteria and the spoilage of milk when the milk is held barely above the freezing point is based on experiments conducted more than thirty years ago on raw milk, it seemed possible that there might be some additional points of interest revealed if the subject were restudied. For example, it seemed possible from a study of the literature that a portion of the chemical changes and the spoilage noted in the early experiments might have been due to the milk enzymes, rather than exclusively to bacteria. This, however, did not prove to be the case; with the exception of some oxidized flavor, the changes in the milk were apparently due entirely to bacteria. The eventual bacteriological spoilage of the milk, marked by extensive proteolysis, was entirely in agreement with the observations of earlier workers.

The only point of importance which appears to be new, though not surprising, is that bacterial growth in pasteurized milk is much slower at 0° C. than in raw milk, so that pasteurized milk, if not recontaminated, keeps from two to three times as long as does raw milk of substantially the same quality or bacterial content. While a good quality of raw milk will usually keep for about four weeks before obvious spoilage occurs, a comparable pasteurized sample usually keeps in excess of eight weeks and sometimes as long as twelve weeks. That the improved keeping quality of pasteurized milk is apparently due to the entire destruction of certain kinds of bacteria is shown by the fact that the reinoculation of pasteurized milk with minute amounts of raw milk decreases its keeping quality to a point substantially the same as that of the raw milk.

The bacteria responsible for the spoilage of milk held just above the freezing point appeared to be mainly, if not entirely, gram-negative, non-spore-forming rods largely of the *Pseudomonas* group. Spore-forming bacteria played no part in the spoilage of the samples of milk studied and, so far as could be determined, made no growth at this temperature.

M4. Thermoduric Bacteria in Milk. III. The Effect of Changing Agar and Temperature of Incubation for Plate Counts on the Problem of Thermoduric Bacteria in Milk. J. L. HILEMAN, CLARENCE MOSS AND BETTY STEAD, Dairymen's League Co-operative Association, Inc., Syracuse, N. Y.

Bacteria counts were made on the old standard agar and on tryptone glucose extract milk agar at both 37° and 32° C. incubation temperatures on 100 lots of milk both before pasteurization and after pasteurization (a) in a commercial plant at 143° F. for 30 minutes, (b) in the laboratory at 143° F. for 35 minutes, and (c) in the laboratory at 161° F. for 16 seconds. The data show that changing the medium from the old standard agar to the new tryptone glucose extract milk agar, or changing the temperature of incubation from 37° C. to 32° C., or making both changes simultaneously, all have the result of increasing counts on both raw and pasteurized milk, when the average of a series of samples is examined. This is in agreement with results published in the literature. However, the percentage increase is far greater in the case of pasteurized milk, being from two to five times as great as with raw milk. This is because there is only a small percentage of thermoduric bacteria among the raw-milk organisms capable of growing on the old agar at 37° C., whereas there is a much greater percentage of thermoduric bacteria among those organisms requiring for their growth any one of the three changes in methods of making counts. This means that the thermoduric problem has been brought to light largely because of the change in the agar. It will be still further aggravated if and when the temperature of incubation is changed.

M5. Effect of Growth of *Pseudomonas putrefaciens* on Aroma Compounds in Butter. P. R. Elliker and B. E. Horrall, Purdue Agricultural Experiment Station.

Observations in the field and laboratory indicated that the development of putrid and cheesy odors and flavors in butter were accompanied, and in many cases preceded, by a loss of aroma in commercial butter. *Ps. putrefaciens* usually was found to be the causative organism. This suggested that *Ps. putrefaciens* might be active in destruction of the aroma compound, diacetyl, present in the butter.

Experimental lots of cream were sterilized in the autoclave, then cooled, and the cream churned in sterile churns. In certain lots the sterile butter granules obtained were then washed with sterile water and in other lots with water contaminated with a pure culture of *Ps. putrefaciens*. The respective types of butter were then worked, sufficient diacetyl added to provide a high aroma in the butter and after thoroughly distributing the diacetyl, the butter was placed in small sterile sample jars. In order to simulate the average treatment of commercial butter, the samples were stored at 70° F. for one week. They were examined at definite intervals for changes in odor, flavor and diacetyl content.

The results show that a definite decrease in diacetyl content accompanied the activity of *Ps. putrefaciens* in the butter during storage when diacetyl was added to the butter. When sterile water was used to wash the butter granules, the loss in diacetyl content in the butter during storage at 70° F. for seven days was very slight. However, when the wash water contained sufficient *Ps. putrefaciens* organisms to produce a cheesy or putrid flavor in the butter, the diacetyl content after four days of storage was reduced to less than one-half the original amount.

M6. The Effect of *Streptococcus agalactiae* Upon the Standard Plate Count of Milk. Max E. Morgan and E. O. Anderson, University of Connecticut.

Strains of *Str. agalactiae* were isolated from 20 different animals occurring in 14 different herds. Pure milk cultures of these 20 strains were plated in parallel on blood agar, Edwards' medium, the new and old standard medium. All 20 cultures grew well on 5 per cent blood agar and Edwards' medium. One culture failed to grow on the old standard medium and another showed no growth on the new standard medium. By using the variance ratio method of analysis, it was found that under the conditions of our experiments there was no significant difference between blood agar and Edwards' medium, and between the new standard and the old standard medium in their ability to support the growth of *Str. agalactiae*.

However, there was a very significant difference in the growth promoting properties of the blood media as compared to the media which did not

contain blood. It was found that the media containing blood supported more colonies than the media without blood in the ratio of $1.284 \overset{\times}{\div} 1.092:1$. Where growth occurred on both Edwards' and the new standard medium, it was found that Edwards' medium supported more *Str. agalactiae* colonies than did the new standard medium in the ratio of $1.375 \overset{\times}{\div} 1.078:1$.

The mean exposed areas of those colonies which grew on the media containing blood were larger than those growing on media which contained no blood in the ratio of $2.191 \overset{\times}{\div} 1.112:1$. A ratio of $4.203 \overset{\times}{\div} 1.184:1$ was found to exist between the mean area of the colonies on the new standard and the mean area of those on the old standard medium.

Data were secured on 94 herds infected with *Str. agalactiae* mastitis. With 33 of these herds in which the per cent of quarters infected was known, a very significant correlation was found to exist between the per cent of quarters infected and the number of *Str. agalactiae* present in the herd samples. Considering all samples, there was no correlation between the total counts on the new standard medium and number of *Str. agalactiae* occurring in the samples. The high contribution of *Str. agalactiae* to the total count was 48.0 per cent, without correcting for the difference in the growth supporting ability of Edwards' and the new standard medium. The logarithmic mean of the total counts on the new standard was 28 per cent higher than the mean of the counts on the old standard medium. In view of the results of the pure culture platings on these two media, it was felt that this difference could not be attributed to the presence of *Str. agalactiae*.

M7. The Lethal Effectivenes of Ultraviolet Rays Applied to Milk. G. C. SUPPLEE, G. E. FLANIGAN, AND O. G. JENSEN, Borden Biological and Chemical Research Laboratories, Bainbridge, N. Y.

The lethal effectiveness of ultraviolet radiation is well-known, but available evidence concerning the degree of destruction of bacteria in milk under conditions which are adaptable for practical use, is very meager. The results from studies with commercial milk extending over a period of some years, have revealed the merits and limitations of this bactericidal principle as applied to milk wherein certain improvements in the experimental technique were employed.

By irradiating smooth flowing milk films of known characteristics and using appropriate spectral quality and intensity of the incident radiation, a reduction in bacteria count of average raw milk of 95 to 98 per cent was obtained, with substantial regularity. This reduction may be accomplished without development of adverse flavor and odor within an exposure period of about seven to eight seconds or less. The spectral characteristics and the intensity of the radiations and method of application were found to be more

significant in obtaining a consistent high percentage reduction, than variations in the resistance of the organisms comprising the usual milk flora.

Sub-lethal applications of ultraviolet energy of which a predominant proportion consisted of short radiation (2200–2300 Å but with none of the 2537 Å line) gave irregular results with evidence that such radiation may actually increase the bacteria count of milk under given conditions. Whether the increase in plate counts was due to a dispersal of clumps or to a stimulating effect on individual organisms is a matter of conjecture.

Irradiation at elevated temperatures, or simultaneous irradiation during elevation of the temperature by electrical heating of the flowing film, did not significantly enhance the lethal effectiveness of the ultraviolet energy; such method of treatment tends to develop a characteristic irradiation flavor.

Percentage reduction curves obtained with an experimental flowing film electric pasteurizer wherein the temperature may be raised to any desired degree within a period of about 0.8 second are compared with percentage reduction curves obtained by ultraviolet radiation under varying conditions of treatment. The data illustrate comparatively, the bactericidal effectiveness of both forms of energy applied to milk under conditions potentially adaptable for other than laboratory demonstration, and wherein the time element is reduced substantially to an irreducible minimum.

M8. Bacteriological Problems in Short Time High Temperature Pasteurization.

HAROLD WAINESS, York Ice Machinery Corpn., York, Penna.

Many dairy plants that have installed a high temperature short time pasteurization system have found that their previous system of laboratory control was inadequate. Neither a raw milk plate count, direct microscopic count, or the methylene blue reduction test, used alone could detect the presence of thermoduric micro-organisms in their present milk supply.

Experiments conducted with a large number of producers in Kentucky, Illinois and Wisconsin have shown that a combination of the resazurin test and plating the milk of individual producers, before and after pasteurization, gave the best results. Here we were able to single out those producers whose milk was high in thermoduric organisms. A visit to their farms always uncovered the source of these organisms in dirty milking machines and utensils.

For small plants that cannot afford expensive bacteriological equipment, the resazurin test used alone was found to be a relatively accurate index of thermoduric contamination.

M9. The Foaming of Milk and Certain Milk Products in Relation to Their Surface-active Constituents.

M. S. EL-RAFEY AND G. A. RICHARDSON, University of California.

Foaming properties of the major surface-active constituents of milk in-

cluding casein, lactalbumin and lactoglobulin are reported. A modified procedure for isolating undenatured casein with a lipid content as low as .06 per cent is used: skim milk is super centrifuged; the casein precipitated at low temperature with 0.1 N HCl; the casein washed, frozen and partially air-dried by an electric fan during thawing.

The foam measuring apparatus used is based on the principle of forcing a known volume of air through a sintered glass disc and the layer of liquid above it, at a definite temperature. According to experimental data, the subsidence of skim milk foams cannot be defined by a single equation at temperatures between 5 and 55° C. A unit called "half-volume time" is recommended, therefore, for comparing the stabilities of foams.

The foaming properties of whey, skim milk, whole milk, and cream have been studied at temperatures between 5 and 55° C. Synthetic solutions containing lactalbumin, calcium caseinate, milk triglycerides and phospholipids have been found adequate to duplicate the foaming properties of these milk products.

The results obtained indicate preferential adsorption of calcium caseinate at the air/skim milk interface at temperatures below 27° C. and of lactalbumin at higher temperatures. Fat has proved to be responsible for the minimum foaming of skim milk at temperatures around 27° C.

A hypothesis supported by experimental work explains the formation of a phospholipid-protein complex that imparts the foaming properties to cream. The formation and stability of a "cream-type foam" has been shown to be a function of the per cent of phospholipid in the fat and the per cent fat in the skim milk or whey emulsions.

M10. Factors Affecting the Gas Content of Milk. C. I. NOLL AND G. C. SUPPLEE, Borden Biological and Chemical Research Laboratories, Bainbridge, N. Y.

A quantitative study has been made of the dissolved gases in milk as affected by light, heat, vacuum, displacement by other gases and processing, in order to observe the general principles governing the gas content of milk. Particular attention has been given to the oxygen content of milk and the study of methods for its removal.

Data are submitted showing that, within the limitations of the experimental procedures used, the oxygen content of milk is primarily a function of the partial pressure of the oxygen over the solution and the temperature of the solution. This is in agreement with the accepted laws of solutions of gases in liquids.

Quantitative experimental data are given showing the gas content of milk at various stages in several pasteurization processes, the loss of gases during the heating and their subsequent reabsorption on exposure to air during cooling.

The degree to which the oxygen content of milk can be lowered by heat (below boiling temperature), vacuum, displacement with other gases, light and light in the presence of added ascorbic acid is presented. The application of these data to the development of methods for the deoxygenation of milk is discussed with appropriate experimental evidence.

The correlated data show that if the dissolved oxygen in milk is completely removed, the vitamin C of fluid or processed milk is stable, notwithstanding subsequent heat treatment or exposure to light. Such factors as heat, exposure to light, the presence of copper, etc., appear to be secondary catalytic influences affecting the rate of destruction of vitamin C only if dissolved oxygen is present.

M11. Factors Influencing the Response of Cream to a Rebodying Process. F. M. Skelton and H. H. Sommer, University of Wisconsin.

It is generally recognized that considerable variation is encountered when subjecting pasteurized cream to a rebodying process such as that devised at the Geneva Experiment Station and known as the Geneva Heat Treatment Method. The wide variations in the response to this method have limited its commercial usefulness. A suitable explanation of the increase in viscosity is lacking, although several have been postulated, *i.e.*, fat globule clumping, increased protein hydration or both.

Recent work at Cornell permits the speculation that a substance "agglutinin" is associated with the fat globule membrane, reversibly adsorbed, and which possesses the property of promoting extensive fat globule clumping as determined indirectly by cream line studies.

It has been demonstrated here in corroboration of the work of Sharp *et al.*, that this substance is adsorbed on solid fat globules, but is released into the skimmilk when the fat is liquefied. With this in mind, experiments were conducted to study the effect of this substance on the viscosity of raw and pasteurized cream and their response to rebodying.

Mixed herd milk was divided into 2 lots. Lot I was separated at cow temperature immediately after milking, while Lot II was first cooled to 40° F. for 6 hours, rewarmed to 90° and then separated. Each lot of cream was standardized with its corresponding skimmilk to 30% fat.

The viscosity of the raw, pasteurized and heat-treated cream from each lot after 24 hours' aging was determined. It is evident from our studies, that cooling prior to separation produces the more viscous raw cream, but pasteurization eliminates this difference. Further, the cream from uncooled milk shows a greater response to rebodying. It would appear then that the previous temperature history of the milk is important in determining the response to rebodying.

Determinations were made of the viscosity of raw, pasteurized and heat-treated cream aged for 24 hours obtained at separating temperatures of 70°,

80°, 90°, 100°, 110°, 120° and 130° F., and also the surface tension values of raw skimmilk obtained at each temperature. Separating temperatures of 80° or 90° F. seem to be near the optimum insofar as response to rebodying is concerned. While the surface tension of the skimmilk was found to increase with the separating temperature, there is no evident relationship to the optimum for rebodying.

Studies were made of the viscosity of raw, pasteurized and heat treated cream prepared as follows: Cream A prepared from milk separated at 60° F. and standardized to 30% fat with skimmilk from milk separated at 130° F. Cream B was prepared from milk separated at 130° F. and standardized to 30% fat using skimmilk from milk separated at 60° F. Cream A was then rich in "agglutinin" while Cream B was supposedly poor in this material. The results substantiate the evidence presented above and lend support to the belief that the substance or substances influencing the response to heat treatment is not inactivated by pasteurizing temperatures, is intimately associated with the fat globule membrane, is reversibly adsorbed being released into the skimmilk when the fat is liquefied.

Preliminary investigations using reconstituted and washed creams indicate that lecithin may be an important constituent of the fat globule membrane materials with respect to its response to rebodying. The addition of lecithin to skimmilk reconstituted cream yields a product which behaves in a somewhat similar manner to normal cream. However, while it must be admitted that such a reconstructed cream is probably far from simulating a normal cream, the results obtained further substantiate and lend support to the belief that substances associated with the fat globule membrane, are important in determining the response to rebodying. Considerably more attention is likewise focused on the temperature history of the milk in view of the fact that changes in the physical state of the fat influence the kind and amount of adsorbed material.

M12. An Improved Micro-Kjeldahl Apparatus and Procedure for the Analysis of Milk. M. C. Rhees, T. R. Freeman, and Chas. N. Shepardson, A. & M. College of Texas.

This progress report concerns improved apparatus and methods developed in an investigation of composition of milk produced in Texas. It was found that certain Kjeldahl technique could be modified to gain considerable speed and accuracy in the analysis of milk.

The results indicate that the improved Micro-Kjeldahl method is readily adaptable for the determination of milk protein, because (1) a .2 gram sample of milk is used in each determination, (2) the use of buffered potassium perchlorate gives a catalyst which effects digestion in 10 minutes, (3) the distilling apparatus eliminates the need for transferring the digested sample, as is necessary with other Micro-Kjeldahl stills, (4) distillation can

be accomplished in three minutes, thus making a complete determination possible within 20 minutes. Duplicate samples checked within .4 per cent of the Official Gunning-Arnold method on milk and whey and recovered over 98 per cent of the nitrogen from urea and potassium ferrocyanide. Where large numbers of samples are to be run it is possible to average a complete determination every five minutes.

Preliminary studies conducted over a one year period showed the milk in Texas to be low in lactose as compared to the average figures reported elsewhere, while the ash is inclined to be higher.

M13. A Progress Report on the Utilization of Apple Products, Especially Apple Syrups and Juices, in Producing Soft-Curd Milk. C. C. FLORA AND C. W. HOLDAWAY, Virginia Agricultural Experiment Station.

There are several methods of lowering the curd tension of market milk but none of these methods have made use of apple syrups or juices as a means of modifying market milk for infant food. The use of apple powder or vinegar (acetic acid) has been suggested for producing certain results in modifying milk for infant feeding, but no curd tension readings were made.

Concentrates of apple syrup, apple juice, apple syrup with increased pectin and apple juice with increased pectin showed considerable reduction in the curd tension of market milk. However, concentrates of apple juice and apple juice with increased pectin produced the greatest influence on curd tension.

Concentrate apple syrup in dilution of 100 ml. of syrup to 300 ml. of market milk lowered the curd tension below 8 grams. Concentrated apple juice in dilutions (1–10) lowered the curd tension to 10 grams or below and concentrated apple juice with increased pectin was almost as effective as the concentrated apple juice. The concentrated apple syrup with increased pectin showed a greater reduction than the concentrated apple syrup, but not as great as with the concentrated apple juice.

The addition of pectin seems to be very effective in lowering the curd tension but the pH seems to be the most important factor.

M14. The Determination of Citric Acid in Milk by the Pentabromoacetone Method. E. F. DEYSHER AND GEORGE E. HOLM, Bureau of Dairy Industry, U. S. Department of Agriculture.

A study of the various procedures of the pentabromoacetone method has been made and the conditions determined which will result in the greatest percentage recovery of citric acid, in pure solution and in milk. The modifications of Lampitt and Rooke have been followed in general, except as follows: (a) After addition of excess permanganate the mixture was allowed

to stand at ice-box temperatures for 16 to 18 hours before discharge of excess permanganate with ferrous sulfate, (b) the precipitate was washed with 50 cc. of water of a temperature of 3° C., and (c) the drying was in a vacuum desiccator at temperatures not over 20° C. With the modified procedure the citric acid in pure solutions was determined with an error of approximately ± 0.50 per cent and in milks with an error of approximately ± 1.00 per cent, of the theoretical values.

M15. The Effect of Flash Forewarming upon the Heat Stability of Evaporated Milk.

B. H. Webb and R. W. Bell, Bureau of Dairy Industry, U. S. Department of Agriculture.

Milk was "flash" forewarmed in a specially constructed tubular heater by heating it in 3 seconds to temperatures up to 163° C. (325.4° F.), maintaining the temperature for 15 seconds and cooling in 2 seconds. Fresh milk generally coagulated during passage through the heater when the temperature was between 160° C. and 163° C. The effect of flash forewarming of milks at different temperatures up to 160° C. upon the heat stability of their evaporated products was determined by sterilizing the concentrated milks in cans at 115° C. until coagulation occurred. The results were compared with the heat stability values obtained by forewarming portions of each milk to 95° C. in accordance with the accepted procedure used in the evaporated milk industry.

Flash forewarming of skim milk at temperatures between 100° C. and 120° C. caused a large increase in the heat stability of its evaporated product as the forewarming temperature was raised. An increase in forewarming temperatures between 120° C. and 155° C. improved stability further. Flash forewarming whole milk up to 120° C. did not improve the heat stability of its evaporated, homogenized product over the 95° C. control. However, increases in the flash forewarming temperature of whole milk from 120° C. to 155° C. caused an abrupt increase in heat stability. The heat stabilities of evaporated skim and whole milks flash forewarmed to their optimum temperatures were increased from 2 to 6 times the stabilities of the control samples forewarmed to 95° C. Among the practical applications of these observations are the possibilities of decreasing the use of stabilizing salts in the manufacture of evaporated milk and of increasing the amount of milk solids which may be included in a can of evaporated milk.

M16. The Influence of Homogenizing Pressures on the "Dryness" of Ice Cream when Drawn from the Freezer.

J. H. Erb and John Whitworth, Ohio State University.

Several series of regular ice cream mixes having the composition of 12 per cent fat, 11 per cent serum solids, 15 per cent sugar, and .25 per cent

gelatin, were processed under varying conditions of homogenization using a Manton-Gaulin two-stage homogenizer. Homogenization was carried out at the pasteurizing temperature of 155° F.

In one series of mixes the pressures were varied from 1,000 pounds to 5,000 pounds single stage. In another series the pressures were the same except that 500 pounds pressure was applied to the second stage. Other trials were run in which the mix was sent through the homogenizer twice at various pressures. The mixes were frozen using a Creamery Package 60 gallon per hour continuous freezer and a York batch freezer. At every trial all mixes were drawn from the freezer at the same temperature, and the apparent wetness or dryness was determined by observing the condition of the ice cream as it emerged from the freezer and also by noting how the ice cream resisted melting when exposed to room temperature for approximately five minutes. The results indicate the following:

(1) The higher the pressure applied to the mix and the oftener it was homogenized, the wetter the ice cream appeared when it was discharged from a continuous freezer at a given temperature. The same difference was noted in the batch freezer, but the differences were not as marked.

(2) The pressure of homogenization was found to have considerable effect on the melting characteristics of ice cream. With mixes of normal acidity and salt balance, the higher the pressure the smoother was the melt down.

(3) Ice cream subjected to high pressures of homogenization or processed at high pressures several times melted at a more rapid rate than mixes processed at lower pressures.

(4) There was found to be a slight improvement in the body of the ice cream processed at the higher pressures.

M17. Monoglyceride-Gelatin as an Ice Cream Stabilizer. P. S. LUCAS, Michigan State College.

Among the promising products perfected during recent years for hastening the incorporation and ease of incorporation of air in ice cream mix during freezing is a homogeneous mixture of a monoglyceride and gelatin. The use of monoglyceride in ice cream is patented. Conceivably, the addition of gelatin and a suitable monoglyceride as separate ingredients to the mix might produce results similar to those produced by a prepared mixture.

In this study both were used: the former at the rate of 0.4 of 1 per cent; and the latter at the rate of 0.3 per cent of gelatin of 275 Bloom grade, with .06 per cent of monoglyceride. Both were checked against gelatin alone, the equivalent amounts of each being calculated from their Bloom test. While emphasis was placed upon the body score of the ice creams, these organoleptic methods were supplemented by microscopic examinations

together with measurements of the ice crystals. Examinations were made at day, week, and two-week intervals.

The gelatin-glyceride mixture reduced freezing time 16 per cent on the average. The score of the product when fresh was increased on the average by about one-third of a point. After one week's storage, the score was reduced to a quarter of a point, remaining at this figure for the two-weeks' storage period. Flavor was affected in no case.

The product made with gelatin and with the gelatin monoglyceride prepared mixture varied more however, than indicated by score alone, the body of the ice cream made with the latter being much more compact and giving the appearance of being heavier and in some cases somewhat richer. These values were paralleled when monoglyceride was added to gelatin at the time of pasteurization in the proportions mentioned. Considerable difficulty was encountered, however, in this case due to the oxidation of the particular glyceride used. This oxidized flavor was carried into the ice cream to such a degree that the product was practically unsalable. This product should not be confused with the prepared mixture of gelatin and monoglyceride.

Microscopic studies showed smaller ice crystals when gelatin and monoglyceride mixture was used. Both products acted in a manner very similar to egg yolk in increasing whippability of the mix.

M18. A Method for the Preparation of Acid Casein for Use in Ice Cream.

L. P. Teichert, T. R. Freeman, W. S. Arbuckle, and Chas. N. Shepardson, A. & M. College of Texas.

This investigation presents a simple and inexpensive method of concentrating skimmilk for satisfactory use as a source of serum solids in ice cream.

Dilute hydrochloric acid was added to skimmilk to form an acid curd. The curd thus formed when washed and drained contained from 29 to 32 per cent solids and could be stored in a frozen condition satisfactorily.

A rapid method of dissolving curd for use in ice cream mix is suggested. This was accomplished by the addition of a basic salt, after which the curd was added to the other ingredients of the mix at a temperature of 120 to 130 degrees Fahrenheit.

The dissolved curd gave most satisfactory results when the pH was adjusted at 6.5 to 7.2.

The adaptability of several neutralizers and the amounts of each necessary to bring the dissolved curd within the desired pH range were studied. Sodium carbonate and sodium bicarbonate proved to be most satisfactory.

A preliminary study indicates that acid casein may be used to supply up to 40 per cent of the serum solids content of an average commercial mix. In most cases the mixes showed excessively rapid whipping ability and a high maximum overrun. In the finished ice cream a slight curd flavor was observed in some cases, and the body was criticized as being gummy.

Results show, however, that the rate of whipping and maximum overrun can be controlled by slightly decreasing the serum solids and gelatin content, or by the addition of small amounts of calicum chloride. The gummy defect was also largely eliminated by reducing the gelatin and serum solids content.

Sandiness was not encountered in any of the ice creams studied even after prolonged holding at fluctuating temperatures.

The data indicate that acid casein prepared from skimmilk may be of considerable value as an economical source of serum solids in ice cream and offers a valuable method of utilizing surplus skimmilk.

M19. The Temperature Method for Control of Whipping in Ice Cream. ALAN LEIGHTON, Bureau of Dairy Industry, U. S. Department of Agriculture.

Experiments show that a maximum whip consistent with the existing temperature is attained in the batch ice cream freezer shortly after the refrigerant is turned off. It follows that, with the position of the temperature-overrun equilibrium line known for the given mix, the thermometer may be used to indicate the proper point for turning off the refrigerant and the proper time for drawing the mix. Such a method is practical only if similar mixes exhibit uniform whipping properties from day to day. The experiments show this to be the case.

The deposition of butterfat upon the blades of the freezer lowers by 5 to 10 per cent the overrun obtainable at a given temperature. Since butterfat usually accumulates slowly upon the blades of a freezer, four or five batches must be frozen before completely uniform results may be expected for the succeeding runs. A drawing temperature of from 0.1° C. to 0.2° C. higher is necessary to overcome overrun loss due to the accumulation of butterfat.

Uniform results have been obtained by applying the method to indentical mixes whipped in a horizontal 20-quart brine-cooled freezer, a 20-quart horizontal full-flood ammonia refrigerated freezer and in a 10-quart vertical freon direct-expansion counter freezer. The time intervals necessary for freezing varied markedly with the different freezers.

By following the procedure outlined in the paper the manufacturer can be sure that he is drawing his mixes at the lowest possible temperature consistent with the desired overrun, also that his freezings are being carried out in the shortest possible time.

M20. Motion Pictures as a Medium for the Study of Ice Cream.* W. H. E. REID, C. W. DECKER, L. E. SMITH, K. R. MINERT, W. S. ARBUCKLE, AND JOE EDMONDSON, Missouri Agr. Expt. Station.

Studies have been made using motion picture photography as a means of

* Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. 736.

showing in detail the effect of several factors upon the physical appearance, resistance to medium high temperatures, and stabilizing properties of ice cream of variable composition and manufacturing methods. This investigation includes the use of variable increments of sucrose and dextrose sugars as influenced by a variation in the freezing procedure and acid standardization of mixes to which are subsequently added bacterial cultures in different volumes.

M21. Homogenization Index as Calculated from Measurements of Fat Globule Size.

ARTHUR W. FARRALL, CHARLES C. WALTS, AND RODNEY L. HANSON, Creamery Package Manufacturing Co.

The comparison and evaluation of the results of homogenization have been expressed as a simple number called homogenization index. This index makes possible the determination quite accurately of even small differences in homogenization.

A microscope is fitted with an eyepiece containing a measuring scale 100 microns square, which is divided into sixteen equal squares, and containing a scale across the center which is divided into 2 micron divisions. The size class, and number of the fat globules exceeding 2 microns in diameter are determined in five random fields, using the oil immersion lens. A chart was constructed to simplify the recording of the data and calculations. The homogenization index is equal to the number of fat globules of two microns in diameter which would have the same volume as the fat globules which exceeded two microns in diameter.

M22. The Effects of the Direct Addition of Carotene and Mixed Tocopherols on the Development of Oxidized Flavor in Milk.

EDWIN B. WILLIAMS AND L. H. BURGWALD, Ohio State University.

Cows in the University herd were selected which produced milk that would develop an oxidized flavor "spontaneously." Part of the milk was pasteurized in glass at 143° F. for 30 minutes and carotene was added directly to the milk both before and after pasteurization at the rate of 500, 1000, and 1500 units of vitamin A per quart. The remainder of the milk was kept raw and carotene was also added to these samples at the same rate as for the pasteurized samples. The source of carotene used was a compound known as Provalac, manufactured by General Biochemicals Company. In no case did the carotene show an inhibitory effect upon the development of the oxidized flavor.

Experiments were conducted to determine the effect of mixed tocopherols upon the development of oxidized flavor in "spontaneous" milk. The milk was pasteurized in glass at 143° F. for 30 minutes. An oil assaying 41.1 per cent of natural mixed tocopherols prepared by Distillation Products, Inc., was added to the milk after pasteurization by emulsifying it into a small quantity of homogenized milk and adding the mixture to the "spon-

taneous'' milk. A concentration of the tocopherol oil of 0.5 per cent of the fat content of the milk was effective in prohibiting the development of the flavor defect for at least 96 hours and in the majority of cases for 144 hours.

A few experiments have been conducted to determine the effect of the tocopherol concentrate upon the development of metal-induced oxidized flavor. Copper was added after pasteurization in the amount of 2 p.p.m. to milk pasteurized in glass at 143 F. for 30 minutes. The tocopherol oil in the concentration of 0.5 per cent of the fat content of the milk inhibited the development of the oxidized flavor up to 72 hours and markedly reduced the intensity of the defect at 96 hours. In one experiment, the tocopherol concentrate completely prevented the development of the oxidized flavor for 144 hours in raw milk contaminated with 2 p.p.m. copper and the pasteurized samples containing the oil had only a trace of oxidized flavor at 120 hours while the control samples had an intensity of 4 plus.

Since the tocopherol concentrate was found to inhibit the development of oxidized flavor, a study was made to determine what effect it had upon the vitamin C content of the milk. The vitamin C content was determined according to the titration method outlined by Sharp. It was found that the addition of the tocopherol oil in the concentration of 0.5 per cent of the fat content of the milk hastened the destruction of the vitamin C in the milk to a marked degree. On the other hand, the addition of carotene at the rate of 1500 units of vitamin A per quart seems to have a very slight protective action towards the vitamin C, although the results are not conclusive.

M23. The Influence of Treated Fibre Milk Containers on the Incidence of Copper-Induced and Sunshine Oxidized Flavors of Milk.

C. L. Roadhouse and J. L. Henderson, University of California.

Manufacturers of fibre milk containers are interested in securing paper stock that will exclude sunlight from milk, and if possible, prevent or delay the development of copper-induced oxidized flavor of milk. Fibre cartons, and strips cut from cartons, were especially treated for use in these experiments.[1]

The containers were treated by two methods: 1. Avenex was added to the paper stock before paraffining to attempt to delay the development of oxidized flavor of milk to be placed in them, and 2. Paper stock was impregnated with titanium dioxide in order to increase its opacity and exclude light rays that might influence the flavor of the milk. The paper strips were prepared by treating the paper stock before paraffining with Avenex, with the n-butyl ester of tyrosine or with cocoa shell flour.

Three types of milk were tested: Holstein, Jersey, and a mixture of the

[1] Fibre containers and strips were prepared by the American Can Company in their plants and laboratories.

two breeds. The milks were pasteurized in stainless steel equipment. In certain experiments 0.25 p.p.m. cupric ion (as copper sulphate) was added when the pasteurizing temperature (143° F.) was reached. After pasteurization fibre containers were filled and the milk flavored daily for three days as unknowns. The fibre strips were tested by being submereged in milk held in half-pint bottles. These samples likewise were scored for flavor daily for three days.

When the influence of sunlight was being studied, the containers were exposed for 30 minutes to the direct rays of the sun during the middle of the day. The milks were tested for ascorbic acid daily for three days after treatment.

Results: 1. Samples that were uncontaminated with copper.

The rate of ascorbic acid destruction in milk exposed to sunlight for 30 minutes in treated and untreated containers indicate no significant differences in the amount of sunlight excluded. In all samples the rate of disappearance of ascorbic acid was slightly greater than in duplicates kept in the dark. In general the milk flavor was only slightly impaired by the 30 minute exposure period.

2. Samples that were contaminated with copper.

None of the treated containers or strips prevented the development of oxidized flavor. The oxidized flavor was more pronounced when the samples were exposed to sunlight for 30 minutes.

M24. An Electric Laboratory Pasteurizer. H. B. Henderson, Thos. B. Harrison, C. E. Wylie, and H. A. Arnold, University of Tennessee.

A pasteurizer was built on the style of the commercial spray type vats. It has six separate compartments or vats making it possible to pasteurize as many as six, two-quart samples of milk at the same time under exactly the same conditions. The milk is heated by a film of hot water flowing down the sides of the vats. This water is electrically heated in a compartment at the bottom of the pasteurizer, and is circulated by a centrifugal pump through specially perforated pipes so arranged as to direct a spray of water against the sides of the vats. The water runs back to the bottom of the pasteurizer where it is reheated and again circulated through the system. The temperature of both the water and the milk is thermostatically controlled. The pasteurizer is of stainless steel construction throughout.

M25. Observations Regarding the Occurrence of Oxidized Flavor in Milk from Individual Cows. H. B. Henderson, W. W. Overcast, and C. E. Wylie, University of Tennessee.

During the past two years considerable data have been collected at the Tennessee Station regarding the development of oxidized flavor in milk from

individual cows as related to feeds, lactation, season, age, level of feeding, and production.

From the data collected it would appear that a high level of feeding may tend to increase the susceptibility of the milk to develop oxidized flavor. This was observed during the summer months, when the cows were on pasture, as well as during the winter months. These data would indicate that lactation, season, age, and feeds may have variable effects on the development of oxidized flavor in milk. Since the milk production follows the general trend of the feeding level it would appear that cows during high production periods may produce milk more susceptible to the development of oxidized flavor, than during periods of lower production.

M26. A Small Electric Holder Type Pasteurizer.* C. W. ENGLAND, ARTHUR P. WIEDEMER, AND GEORGE J. BURKHARDT, Maryland Agricultural Experiment Station.

The enactment of ordinances prohibiting the sale of raw milk to the consuming public has created a demand in rural communities for a small pasteurizer. This pasteurizer studied consists of a rectangular synthetic rubber-lined steel vat of 12 gallons capacity with an agitator of non-conducting material and electrically heated with a carbon electrode in each end. The resistance of milk to the flow of an alternating current between the electrodes provides heat for pasteurization.

To compare the efficiency and adaptability of the electric pasteurizer, various lots of milk were pasteurized in a 200-gallon, spray-type holder pasteurizer and in the electric pasteurizer, and results of tests on each compared. Tests made on each lot of milk included: flavor score, creaming ability, total bacteria count, coliform count, per cent butterfat, per cent titratable acidity, pH and curd tension. The results of these tests indicate that the process used in the electric pasteurizer is essentially a heating process and that when like temperatures and holding periods are used, the results of the electric and spray-type holder methods compare favorably.

Tests have also been made to determine the practicability of pasteurizing uncooled milk. The results of these tests show that pasteurization of uncooled milk immediately after milking compares favorably with milk cooled prior to pasteurization.

The time required for heating small batches is somewhat greater and the efficiency is somewhat lower than for large batches. Approximately 0.24 kwhr. is required during the holding period regardless of the size of the batch. Using current at 2 cents per kwhr., the total current cost of pasteurizing a 12-gallon batch would range from 3.96 cents with a starting temperature of 90° F. to 7.32 cents with a starting temperature of 38° F.

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M27. Some Factors Influencing the Quality of Cream Cheese. B. M. ZAKARIASEN AND W. B. COMBS, University of Minnesota.

The following standard manufacturing procedure served as a foundation from which other manufacturing procedures were developed in an attempt to improve the firmness, texture, shrinkage, keeping quality and flavor of cream cheese:

Cream was standardized at 16 per cent butterfat, heated to 150 degrees F., run through a single stage homogenizer at 1500 pounds pressure and then cooled to 70 degrees F. Forty-hundredths per cent of active starter was added and the cream held at 70 degrees F. until a titratable acidity of .70 to .75 per cent was developed. The clotted cream was agitated while being heated to 120 degrees F., held for 30 minutes and then an equal quantity of cold water was added after which it was cooled to 65 degrees F. The clotted cream was then poured into muslin bags and pressed to one-half the weight of the original cream while being held at room temperature. The drained curd was then placed in a steam jacketed kettle and both salt and gelatin added at the rate of .75 per cent. It was then heated to 150 degrees F., homogenized at 3000 pounds pressure and allowed to flow into the containers.

Some of the factors that were studied are as follows:

A. Variations in treatment of the cream.
   1. Varied the heating temperatures from 150 to 175 degrees F., homogenization temperatures from 150 to 175 degrees F. and pressures from 1500 to 3000 pounds.
   2. Varied the titratable acidity in the ripened cream .65, .85, .90 and 1.0 per cent.
   3. Varied the temperature at which the ripened cream was cooked 120, 140 and 160 degrees F.
   4. Added water before cooking ripened cream.
   5. Eliminated the practice of adding water to the ripened cream.
   6. Eliminated the practice of homogenizing the cream after pasteurization and before the addition of the starter.

B. Variations in the treatment of the cheese.
   1. Varied the temperatures from 150 to 175 degrees F., homogenization temperatures from 130 to 175 degrees F. and pressures from 1500 to 4000 pounds.

The greatest improvement in the quality of the cream cheese was shown when the cream and the cheese were homogenized at relatively high temperatures and pressures, namely, 175 degrees F. and 4000 pounds pressure.

Homogenization of the cream for cream cheese reduced fat losses and prevented the oiling off of the butterfat during the heating of the cream cheese previous to homogenization.

A fine mild flavor was secured in the cream cheese when the cream was ripened to about .70 to .80 per cent titratable acidity.

No practical improvement was noted in the quality of the cream cheese when the ripened cream was heated above 120 degrees F., when the water was added to the ripened cream before heating instead of after or when the addition of the water to the ripened cream was entirely eliminated.

M28. A Short Method of Making a Soft Cheese Similar to Cream Cheese.

E. L. Reichart and L. K. Crowe, University of Nebraska.

Cream cheese as made commercially today, usually contains from 30 per cent to 40 per cent fat and from 40 per cent to 50 per cent total solids. It is made by coagulating milk and cream mixtures, through the development of acidity, by means of starters, with or without the addition of rennet, heating and draining the curd, pressing to expel additional moisture, and packing, hot or cold, in a final container. This process usually requires from 48 to 72 hours, considerable equipment and space, and produces a finished product which in many cases is quite perishable.

A soft cheese of similar composition, of equally good flavor and texture characteristics and of superior keeping quality can be made by the following short-time method. Cream, cottage cheese curd, and salt, are used in such proportions as to give the desired fat and total solids content. This mixture is heated to 180° F., homogenized, and packaged directly from the homogenizer in the desired final package, and allowed to cool at a temperature of 35° to 40° F.

In these experiments, 150 batches of cheese were made from mixtures consisting of sweet and sour cream, cottage cheese curd, condensed skimmilk, and superheated condensed skimmilk, in such combinations as to vary the composition and to note the effect of these variations on the flavor and body of the finished product. Titratable acidities, pH values, and temperatures and pressures of homogenization were also varied to note the effect of these variations on the finished product.

It was found that mixtures of ripened cream (65 per cent fat, and .4 per cent–.45 per cent titratable acidity), cottage cheese curd, and salt, standardized so that the finished cheese would contain 30 per cent–32 per cent fat and 43 per cent–46 per cent total solids, and a pH of 4.5–4.7, heated to 180° F. and homogenized at 2500 lb. pressure, gave a finished product that had the most desirable flavor, body and texture characteristics, as well as the best keeping quality and compared favorably with similar products found on the market at the present time.

M29. A Survey of Commercial Cottage Cheese.

Milton J. Foter, E. O. Anderson, and L. R. Dowd, University of Connecticut.

A survey was made of open-market and plant samples of cottage cheese. The microbiological analysis consisted of determining the relative numbers of bacteria, molds and yeasts per gram of cheese and the presence of members of the coliform group. The cheese was analyzed for flavor, texture, fat, salt and moisture.

The microbiological analysis showed the numbers of bacteria to vary widely. The most common spoilage organisms found were yeasts varying in numbers from 0 to 24,000,000 per gram of cheese. The flavor analysis showed that 31 per cent of the total number of samples were yeasty, 22 per cent sour and 20 per cent bitter. Only about 10 per cent of the samples were not criticized for flavor. The fat, salt and moisture content of the cottage cheese examined varied from 0.88 per cent to 10.8 per cent; from 0.06 per cent to 1.0 per cent and from 67 per cent to 84.5 per cent, respectively.

The survey indicates a need for a more complete study of the yeast and mold flora, with respect to the establishment of standards for cottage cheese.

M30. The Relationship of Acidity to the Quality of American Cheddar Cheese.

H. L. Wilson, S. A. Hall, and H. R. Lochry, Bureau of Dairy Industry, U. S. Department of Agriculture.

Laboratory investigations show that in making Cheddar cheese from raw milk of inferior quality a relatively high acid development is advisable, but if the milk is of good quality or is pasteurized the acidity at milling should not exceed pH 5.40 and the rate of development should be slow. In applying this to factory practice 183 vats of cheese were made in 2 average small factories. The milk was graded on the methylene blue test, pasteurized, the acidity at milling held to pH 5.40–5.55 with a making time of 4½ hours. Duplicate samples were stored 6 months at 34° and 50 or 60° F. and graded.

Of the cheese made in factory number 1, 53.9 per cent of the cheese stored at 34° F. and 7.8 per cent of the cheese stored at 60° F. scored 92 or better. In factory number 2, all cheese stored at 34 and at 50° F. scored 92 or better.

The cheeses from factory No. 2 were remarkably uniform, and samples at 50° F. were scored, on account of more flavor, an average of ½ point higher than those at 34°.

These experiments show that milk of good quality, pasteurized and made with proper control of acid development, will cure satisfactorily and develop a fine flavor at 50° F.

M31. Keeping Quality of Butter Stored at Low Temperature for Six Years.

B. J. Scheib, E. S. Guthrie, and C. N. Stark, Cornell University.

Previously we have reported on the effect of certain factors upon the keeping quality of butter, when the butter was held at a temperature which would permit the growth of bacteria present. We have also reported on the growth of certain fat-splitting and casein-digesting bacteria present in butter in sufficiently large numbers to be responsible for the observed deterioration of the butter. Attention has been called to the possible significance of the presence of even small numbers of certain protein-digesting bacteria,

because of their ability to grow in butter, and the value of milk agar for detecting these bacteria in fresh butter has been stressed. Knowledge of the physiology of these proteolytic bacteria and also practical experience in buttermaking indicate that recontamination of pasteurized cream is usually the source of these bacteria.

Samples of these same batches of butters (96 in number) have been held at $-10°$ F. for six years. Since bacteria are unable to grow at this temperature, it has been possible to observe the effect of (1) temperatures of pasteurization of cream, (2) salt, (3) acidity, and (4) salt and acidity upon the keeping quality of butter. The results of this study show the individual and combined effect of these factors on butter quality. Pasteurization at 165° F. for 30 minutes destroyed the harmful natural enzymes in cream; 145° F. for 30 minutes did not. The presence of salt definitely lowered the quality of the butter; butter made from acid cream was of poorer quality than sweet cream salted butter; the presence of both salt and acid produced butter of still lower quality. The average scores for butter, made from cream pasteurized at 165° F. for 30 minutes, and held at $-10°$ F. for six years were: sweet cream butter 92.3; sweet cream butter salted 90.8; sour cream butter 87.5; sour cream butter salted 85.4.

M32. Mold Mycelia in Cream.* E. R. GARRISON AND J. H. GHOLSON.

This investigation was made to study the influence of farm methods on the amount of mold mycelia in cream. Beginning in October, 1940, samples of cream were obtained once every three weeks at each of the four cream stations in Columbia. The name of the producer, weight of cream, per cent butterfat, flavor, cream grade, temperature of the cream and days since the last delivery were obtained from the cream station operator. Samples of cream were obtained altogether from 310 producers. Questionnaires on farm methods of handling cream were filled out by personal interviews with 80 producers. The sources of mold on the farm were studied by examining the utensils by the agar disc and cotton swab methods and by plating feeds and other materials on acidified potato dextrose agar.

The cream samples were placed in ice water when collected and later taken to the laboratory where the following determinations were made: titratable acidity, formol titration, modified Wildman MBB (methylene blue-borax) test and the plate count for mold on acidified potato dextrose agar. A microscopic examination was made of each sample that had a low mold count and a doubtful or excessive MBB rating.

The grade of 655 cream samples as given by the cream station buyer and the MBB ratings were proportioned as follows: *Grade No. 1,* Good 44.0 per cent, Fair 21.0 per cent, Doubtful 7.0 per cent, Excessive 4.0 per cent. *Grade No. 2,* Good 2.3 per cent, Fair 3.5 per cent, Doubtful 2.2 per cent,

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Excessive 1.1 per cent. *Grade No. 2 (test)* consisting of cream with a butterfat test of less than 25 per cent and not Grade No. 2 because of other defects had MBB ratings of Good 6.6 per cent, Fair 6.3 per cent, Doubtful 1.5 per cent, Excessive 1.1 per cent.

The index of correlation between the titratable acidities and MBB ratings was found to be +.822 with the standard error of estimate being .124 per cent. This correlation is very significant and indicates that in cream with high acid it is very probable that considerable mold is present.

The index of correlation between the MBB ratings and the logarithms of the mold plate count was found to be +.6813 with the standard error of estimate being 1.1640. While this correlation is significant it indicates that there are probably other factors besides the amount of mold mycelia in the cream that affects the MBB rating. The microscopic examination of those samples with a doubtful or excessive MBB rating and a low mold count showed the presence of many body cells which may have been due to either infected udders or cows in late lactation.

M33. Effect of Udder Infection and Late Lactation on the Methylene Blue-Borax Test for Mold Mycelia in Cream.* E. R. GARRISON AND J. H. GHOLSON.

It is generally assumed that the amount of sediment obtained by the MBB (methylene blue-borax) test is proportional to the amount of mold mycelia in the cream. However, occasional samples of cream that give a fair, doubtful or excessive sediment test according to the American Butter Institute mold standard contain very few mold by the plate count on acidified potato dextrose agar. A microscopic examination of the sediment obtained from the MBB test on these cream samples usually shows the presence of many body cells but few or no mold mycelia. This indicated that udder infection or late lactation might be factors that affect the MBB test on producers' cream.

In order to obtain information on this problem the milk from 72 individual cows in the University Herd was studied. The pH, per cent chlorides, and number of body cells in the fresh milk was determined. The milk was then held at 35°–40° F. for 24 hours then the cream was siphoned off and the MBB test performed on the cream and the skimmilk. The sediment test on the cream from 34 cows was rated as good, 20 as fair, 11 as doubtful, and 7 as excessive. The skimmilk gave very little or no sediment by this test even when the sediment test on the cream from the same sample was doubtful or excessive. The cream-mold reagent mixture from many samples was thick and slimy and difficult to filter. A microscopic examination of the sediment usually showed the presence of many body cells but no mold mycelia. There was a general, but not a close correlation, between the num-

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ber of body cells in the milk and the amount of sediment from the MBB test on the cream. Centrifugally separated cream always gave a good MBB test even when the test on the gravity cream from the same milk sample was doubtful or excessive. This was probably due to the removal of many of the body cells and much of the mucous protein during centrifugal separation of the milk.

When the MBB test was applied to the gravity separated cream from the milk from individual quarters of the udder the amount of sediment varied with the different quarters and was correlated with the severity of infection. Many of the cream samples that gave a positive MBB test were from cows in advanced lactation with no udder infection. There was no variation in the MBB test with the individual quarters of these cows and the test became negative after the cows again freshened.

M34. The Effect of Various Factors on Mold Mycelia in Cream and Butter.* W. H. E. Reid, Joe Edmondson, and W. S. Arbuckle.

This investigation is concerned with various factors affecting mold mycelia development in cream and butter. Among these factors are the effect of time, variable temperature, stirred and non-stirred samples, variable butterfat percentages, layered and non-layered samples, and covered and non-covered samples.

Mold mycelia tests were made of the cream by the application of the methylene blue borax (MBB) test which is a modification of the Wildman method. The MBB and the microscopic count were used in the butter analysis. The cream and butter samples were also analyzed for acidity, pH and organoleptic factors.

The data show that temperature is an important factor in the growth of mold mycelia in cream; it multiplies rapidly at the higher temperatures. This particular mold tolerates high acid. There is also a direct correlation of the time factor with the development of mold mycelia. The development of acidity, as the storage time was prolonged, may also have favored mold growth.

In a comparison of the stirred and non-stirred samples of cream the mold growth was more apparent in the stirred samples. This may be explained by the fact that this mold required air for development and grows only on the surface. In the resultant butter the non-stirred samples had a higher count because in the stirred samples the mycelia were broken up to such an extent that they could not be counted as positive fields.

Studies on covered and non-covered, layered and non-layered and variable butterfat series are in progress and the data are not sufficiently complete for conclusion.

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THE FEEDING VALUE OF GRASS SILAGE IN THE RATION FOR DAIRY COWS

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The ensiling of legumes and grasses has been practiced to a limited extent for years, although the practice has not been generally accepted because of recurrent failures. Recent investigations have shown possible methods of preventing these failures. The ensiling of legumes and grasses by these methods has been spurred on during recent years by extension workers, interested in the production of better quality roughage, and by commercial companies, interested in the sale of preservatives or equipment used in the process of ensiling.

The terminology with reference to ensiled crops is not uniform. Throughout this report the terminology of Bender and Savage (2) will be used. They used the term "grass silage" as an all-inclusive term referring to any "silage made from an uncured hay crop—whether it be a true grass such as timothy, a legume such as alfalfa, or a green cereal such as oats. Terms like 'alfalfa-molasses silage' or 'timothy-phosphoric acid silage' describe specific kinds of grass silage."

Among the feeding experiments with grass silage, Watson and Ferguson (15) have made extensive reports. They fed respective groups of dairy cows A.I.V. silage, molasses silage, and artificially dried grass. A statistical analysis of the milk production, butterfat production, and live weight changes showed no significant difference when equal amounts of starch equivalent and digestible crude protein were furnished in each ration.

A comparison of alfalfa silages prepared by the A.I.V. and molasses methods was made by Bohstedt *et al.* (3) in which feeding trials were carried out for three years on milking animals. During the three years the milk and butterfat production records were maintained almost equally well, although the molasses silage had a slight advantage. Each animal received 1 oz. of $CaCO_3$–Na_2CO_3 mixture (10:3) per 15 pounds of silage.

Bender *et al.* (1) have shown that molasses grass silage will replace corn

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silage or hay in the ration of a dairy cow without influencing the production level of the cows to a marked extent. When fed in place of both corn silage and hay, the production level was maintained, although there was a slight loss in body weight. It was believed that a slight increase in the grain ration could have overcome the slight loss in body weight manifested by the animals fed grass silage as the only roughage.

Alfalfa hay grown on irrigated land was compared with mixed grass and clover hay and grass silage by Hodgson and Knott (6). They fed a concentrate mixture according to production. The cows on the mixed hay and silage ration consumed 78.6 per cent as much dry matter as the alfalfa hay group. The cows fed alfalfa hay lost only 33 per cent as much live weight as the cows receiving the mixed hay and grass silage ration. The production of milk for the cows on the mixed hay and grass silage ration was 94.2 per cent as much as for those on the alfalfa ration.

Hayden *et al.* (5) fed two groups of milking cows A.I.V. alfalfa silage and alfalfa hay respectively. By a reversal method they found no significant difference in the production of the animals in the two groups. The A.I.V. silage was supplemented with four ounces of ground limestone per cow per day.

Many other feeding experiments (8, 11, 12, 13, 14) have been conducted to determine the relative value of different silages, different plants in dry or ensiled form, and different combinations of these plants. These experiments were usually of short time duration and, almost without exception, showed no significant difference between the various feeds when they were of good quality and the dry matter intake of the animal is constant.

The work here reported from the Cornell University Experiment Station covers four feeding experiments with milking animals. These experiments were designed to compare grass silage preserved with phosphoric acid with grass silage preserved with molasses, and to determine their value in replacing varying amounts of corn silage and dry hay.

The general procedure for all experiments included complete feed and production records. The animals were milked and fed twice daily. The gain or loss of body weight was determined from the average of three daily weights of the cows, taken at the beginning and end of each period in all experiments.

The chemical analyses of all feeds and the digestion coefficients used in calculations are given in tables 4 and 5. The analysis of the grain mixture was computed from Morrison's tables (9). This computed analysis was used in determining the consumption of feed nutrients in the grain mixture.

EXPERIMENT I

In this experiment the basic ration of one pound of dry hay and three pounds of corn silage per hundred pounds of body weight was varied by the

replacement of a part of the corn silage or dry hay, or both, with grass silage, and the effect on production and body weight determined. A triple reversal experiment was planned with five milking animals in each of three groups, A, B, C. The groups were balanced as closely as possible in milk production, fat test, body weight, breed, age, date bred, and condition.

The three rations used were as follows:

Ration I:

Corn silage—3 pounds for each 100 pounds live weight.
Mixed hay—1 pound for each 100 pounds live weight.
Grain—an 18 per cent concentrate mixture according to production.

Ration II:

Corn silage—3 pounds for each 100 pounds live weight.
Mixed hay—$\frac{1}{3}$ pound for each 100 pounds live weight.
Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to $\frac{2}{3}$ pound of mixed hay) for each 100 pounds live weight.
Grain—an 18 per cent concentrate mixture according to production.

Ration III:

Corn silage—2 pounds for each 100 pounds live weight.
Mixed hay—$\frac{2}{3}$ pound for each 100 pounds live weight.
Molasses silage (grass, clover and alfalfa)—2 pounds (equivalent to $\frac{2}{3}$ pound mixed hay) for each 100 pounds of live weight.
Grain—an 18 per cent concentrate mixture according to production.

The corn silage was well-eared and at a medium stage of maturity.

The grass silage was preserved with 41.75 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses—40 per cent; clovers—30 per cent; alfalfa—20 per cent; weeds—10 per cent. The crop was cut early and stored in the silo with a minimum of drying. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The dry hay was mixed grasses, clovers, and alfalfa of good quality, green and early cut. It was unofficially graded as U. S. No. 1 mixed hay. It was selected as being comparable to a type of hay that could have been made from the green crop used for grass silage under desirable weather conditions.

The grain used was a regular 18 per cent protein commercial open formula mixed feed as follows:

480 lbs. corn gluten feed
200 " wheat bran
100 " hominy feed
220 " ground oats
220 " coconut oil meal
300 " corn distillers dried grains

100 " soybean oil meal (hydraulic or expeller process)
160 " ground barley
180 " cane molasses
20 " steamed bone meal
20 " common salt

The three rations were fed to the respective groups in the following sequence:

	Ration I	*Ration II*	*Ration III*
First 5-week period	Group A	Group B	Group C
Second 5-week period	Group C	Group A	Group B
Third 5-week period	Group B	Group C	Group A

In determining results the feed consumed and milk produced were totalled from the three groups on each ration and reduced to the basis of one cow for one day. These final data are given in table 1.

This table indicates that the production of the animals on the different types of rations was practically the same. The greatest difference in daily average production of 4 per cent fat-corrected milk was 1.2 pounds. This is not a significant difference. The animals on Ration I had a slight advantage in that they received more total digestible nutrients. This caused a gain in weight of the animals on this ration.

EXPERIMENT II

The effect of continuous feeding of molasses grass silage as the only roughage was studied. Ordinary dairy practices call for both a succulent and a dry feed, and it is usually supposed that a combination of feeds is advantageous over a single feed.

A continuous 12-week feeding experiment was planned. Eleven animals were selected which were in fairly heavy milk production, but had passed their peak of lactation. All animals were less than three months pregnant at the end of the experiment. All of the animals were in good and thrifty growing condition. Animals of various breeds and body weights were used in the experiment.

The maximum amount of molasses silage (grass, clover and alfalfa) that the animals would consume was fed. The body weights and the condition of the animals were very closely watched and enough grain was fed to maintain both normal milk production and body weight. It was assumed that the animals' milk production should not decline faster than a normal production curve, and that they should gain weight slightly during advancing lactation, or at least maintain weight during the period of this experiment.

Molasses silage (grass, clover and alfalfa) was fed from the same silo as in Experiment I. Each animal was fed as much of this silage as it would consume. The grain was of the same mix as that used in Experiment I. An attempt was made to feed it in such quantities that the milk production and body weights be maintained in a normal way.

TABLE 1

Daily average feed consumption, nutrient consumption, weight change, and production of animals on the various rations of Experiment I, II, III, and IV

	Grain	Dry hay, lb.	Molasses grass silage, lb.	Phosphoric grass silage, lb.	Corn silage, lb.	T.D.N. fed, lb.*	Digestible protein fed, lb.*	Milk produced, lb.	Butterfat percentage	Fat produced, lb.	Production 4% fat-corrected milk, lb.†	T.D.N. required for maintenance and production‡	Digestible protein required, lb.‡	Excess T.D.N.	Excess lb. digestible protein	Weight change lb.
Daily average per cow for Experiment I (3 periods)																
...	7.7	11.2	....	....	33.4	16.4	2.22	26.5	3.66	.97	25.1	16.9	1.95	– .5	+ .27	+.9
...	7.6	3.7	21.1	....	33.1	14.9	2.06	26.7	3.70	.99	25.5	17.1	1.97	–2.2	+ .09	+.2
...	7.9	7.3	20.9	....	22.2	15.6	2.20	28.0	3.58	1.00	26.3	17.3	2.01	–1.7	+ .19	+.3
Daily average per cow for Experiment II																
	16.44	....	71.77	....	....	21.33	3.60	43.04	3.83	1.65	41.96	22.82	2.81	–1.49	+ .79	+.0
Daily average per cow for Experiment III (2 periods)																
...	10.2	....	28.7	....	33.9	19.7	3.1	28.8	4.26	1.23	29.93	18.4	2.2	+1.4	+ .9	+.0
...	10.2	....	....	32.9	32.3	19.4	2.5	27.9	4.33	1.21	29.32	18.2	2.1	+1.2	+ .4	+.0
Daily average per cow for Experiment IV (2 periods)																
...	13.87	10.28	....	37.32	....	22.6	3.7	36.9	4.13	1.52	37.58	20.4	2.5	+2.3	+1.1	–.3
...	13.78	9.81	29.49	....	....	21.9	4.1	36.6	4.09	1.49	37.06	20.7	2.5	+1.2	+1.6	–.4

alculated from average of analyses made during experiment (tables 4 and 5).
alculated according to Gaines (4).
alculated according to recommended standards by Morrison (9) for each animal and totalled.
ight used was average of initial and final weights for period.

Table 1 gives records of the feed consumed, nutrients consumed, weight change, and production of the animals on Experiment II. This shows clearly that a very constant weight was maintained. This, however, was accomplished with a rather high rate of grain feeding. This high rate of grain feeding was necessary in order to make up for the lack of total digestible nutrients consumed in the roughage part of the ration.

Because this was a continuous feeding experiment with no control group, the production of these animals must be compared with normal.

Landis (7), working with Savage, plotted 389 lactations of animals from three large herds. These animals gave, on the average, a 2 per cent decline per week between the peak of lactation and the fifth month following breeding. Because these records were largely taken from experiment station herds, the normals derived should be comparable to those used in the experiment.

Table 2 gives the average daily production for each week of the 11 animals, and their calculated production (with a normal 2 per cent per week decline) according to Landis and Savage. It is indicated clearly in table 2 that the production of animals on the continuous feeding of molasses silage (grass, clover and alfalfa) was maintained during the duration of this experiment.

TABLE 2

A comparison between the actual production and normal expectancy of production per cow per day for animals on experiment II, on continuous feeding of molasses silage (grass, clover and alfalfa) as the only roughage

Week	Actual daily production*	Actual per cent decline	Expected normal daily production†
Initial	44.55	......	44.55
1	44.29	0.6	43.66
2	45.97	−3.8	42.79
3	45.76	.5	41.93
4	43.81	4.3	41.09
5	43.19	1.4	40.17
6	42.73	1.1	39.37
7	41.68	2.4	38.58
8	39.97	4.1	37.81
9	40.82	−2.1	37.05
10	38.89	4.7	36.31
11	38.26	1.6	35.58
12	38.26	0.0	34.87

* Four per cent fat-corrected milk calculated according to Gaines (4).
† Calculated according to Landis (7) (2 per cent decline per week).

As this experiment was set up for ad libitum consumption of grass silage, it is significant to note the variation in consumption of grass silage by the different animals. This is shown as daily consumption of grass silage per animal per 100 pounds of live weight. These data, with the grain to milk ratio, are given in table 3 for each animal.

It is evident from the data in this table that the rate of consumption

among individual animals is very variable, and that, when the consumption per 100 pounds of body weight decreases, the grain to milk ratio must increase inversely in order to supply the necessary feed nutrients.

TABLE 3

Illustrating high grain feeding necessary to maintain production and body weight on a continuous feeding of molasses silage as the only roughage

Breed	Lb. grass silage consumption per 100 lb. live weight	Lb. of 4 per cent fat-corrected milk produced daily*	Lb. of 4 per cent fat-corrected milk produced for each lb. of grain fed*
Holstein	6.61	57.90	2.84
Ayrshire	6.02	36.49	2.38
Holstein	5.29	54.14	2.58
Holstein	6.13	38.35	2.26
Jersey	5.64	33.05	2.34
Holstein	3.87	48.99	2.13
Holstein	6.18	40.77	2.71
Ayrshire	4.68	29.82	2.21
Holstein	7.91	38.43	2.38
Guernsey	7.63	38.92	3.31
Shorthorn	5.96	44.75	3.25
Actual average	5.99	41.96	2.55

* Calculated according to Gaines (4).

EXPERIMENT III

The use of phosphoric acid was proposed for the preservation of green grass by Wilson (16). Because of the widespread publicity given to this method by commercial companies, and its adoption by many farmers, a single reversal feeding trial was planned to compare the value of a green crop preserved with molasses and one preserved with phosphoric acid when both were fed with corn silage and no hay.

The twelve milking animals selected for this experiment were divided into two balanced groups.

The rations used for this group of animals are designated as Rations I and II. They are as follows:

Ration I:
- Corn silage—3 pounds for each 100 pounds live weight.
- Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.
- Grain—an 18 per cent concentrate mixture according to production.

Ration II:
- Corn silage—3 pounds for each 100 pounds live weight.
- Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.
- Grain—an 18 per cent concentrate mixture according to production.

The corn silage was made from a crop that was well-eared and at a medium degree of maturity.

The molasses grass silage was preserved with 48.06 pounds of blackstrap feeding molasses per ton of green crop. The approximate per cent of each crop in the silage was as follows: grasses, 35; clovers, 40; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. It was, however, felt that water would increase the value of the silage. Therefore, a three-fourth-inch stream of water was entered at the throat of the chopper. After the usual spoilage at the top, the rest of the silage came out in excellent condition.

The phosphoric acid grass silage (grass, clover and alfalfa) was preserved with 27.5 pounds of 68 per cent (food grade) phosphoric acid per ton of green material. The approximate per cent of each crop in the silage was as follows: grasses, 50; clovers, 25; alfalfa, 20; weeds, 5. The crop was cut early and stored in the silo with a minimum of drying. Water was added to this grass silage as it was to the molasses grass silage with a three-fourth-inch stream at the throat of the chopper. There was considerable spoilage at the top and around the edges through the entire depth of the silage. This was believed to be due to air leakage through a poorly constructed silo wall. Only good silage was selected and used to feed the experimental animals.

The grain mixture fed to both groups was a special 18 per cent protein, commercial, open formula mixed feed, as follows:

400	lbs.	wheat bran
400	"	ground corn or hominy
370	"	ground oats
300	"	fresh coconut oil meal
300	"	corn distillers' dried grains (not less than 10 per cent fat)
200	"	linseed oil meal, O.P.
20	"	steamed bone meal
10	"	common salt

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These final results are given in table 1.

These indicate that, with the conditions as represented in this experiment, there was little difference between the two rations. That is, grass silages preserved with molasses and grass silages preserved with phosphoric acid were equal in value as a supplement to corn silage.

EXPERIMENT IV

This experiment was designed much the same as Experiment III. Grass silage preserved with phosphoric acid was fed to one group, and grass silage preserved with molasses was fed to another group. The one important dif-

ference is that dry hay was used to supplement the grass silage, instead of corn silage. The object of the experiment was to compare the value of the two silages when fed with dry hay.

The ten milking animals selected for this experiment were divided into two balanced groups, A and B.

The rations were as follows:

Ration I:

Mixed hay—1 pound for each 100 pounds live weight.

Phosphoric acid silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture, according to production.

Ration II:

Mixed hay—1 pound for each 100 pounds live weight.

Molasses silage (grass, clover and alfalfa)—3 pounds (equivalent to 1 pound mixed hay) for each 100 pounds live weight.

Grain—an 18 per cent concentrate mixture according to production.

The mixed hay was early cut and well cured. It was unofficially graded as follows: U. S. Grade No. 2 mixed hay. It contained timothy, red top, quack, alfalfa, blue grass, and red clover. Both the molasses and phosphoric acid grass silage were from the same silos as that used in Experiment III. The grain used was of the same mix as that used in Experiment III.

In determining results the feed consumed and milk produced were totalled from the two groups when on each ration and reduced to the basis of one cow for one day. These data are given in table 1.

These indicate that, with the conditions as represented in the experiment, there was little difference between the two rations. That is, grass silage preserved with molasses and grass silage preserved with phosphoric acid were equal in value as a supplement to mixed hay.

DISCUSSION OF RESULTS

Throughout these feeding experiments it was evident that the palatability of the silage depended on the quality of the crop ensiled, moisture content, and the preservative added.

These experiments indicate clearly that the production of the animals was not significantly different on the various combinations of roughage, providing the total nutrient intake remained constant.

When molasses grass silage was fed as the only roughage ad libitum in Experiment II, the consumption of nutrients in the form of roughage varied considerably with individuals. The average was 5.99 pounds of grass silage per hundred pounds, which furnished nearly the normally expected intake of nutrients in the form of roughage. The range, however, was from 3.87 to 7.91 pounds per hundred pounds live weight, which means that many animals will not consume so large an amount of dry matter in the form of

molasses grass silage as in combinations of more than one roughage as a succulent and dry feed. This necessitates the feeding of a greater amount of concentrates. In this regard it is significant to note that there was a great range in the rate of grain feeding. Certain animals consuming a small amount of grass silage were fed on a narrow grain ratio (as narrow as one pound of grain to 2.13 pounds of 4 per cent fat-corrected milk), while the others consuming a large amount of grass silage were fed on a wider grain ratio (as wide as one pound of grain to 3.25 pounds of 4 per cent fat-corrected milk). The average was one pound of grain to 2.55 pounds of 4 per cent fat-corrected milk. This was the narrowest grain to milk ratio of any group of animals included in the feeding experiments.

Although the production and body weights were satisfactorily maintained on a continuous feeding of molasses grass silage as the only roughage, it would not seem a practical feeding practice because of the decreased nutrient consumption in the form of roughage.

From Experiments III and IV there is evidence that the kind of preservative did not affect the feeding value of the silage, providing the total intake was maintained. There is also evidence to indicate that either phosphoric acid or molasses grass silage is a satisfactory supplement to corn silage or dry hay when fed under conditions as represented in these experiments.

When grass silage is used to replace all the dry hay, the ration may be deficient in vitamin D, and precautions should be taken against this deficiency when this feeding practice is continued over long periods. Similarly, when grass silage is used to replace corn silage, the nutrient intake may be lowered because of the lower percentage of total digestible nutrients in grass silage as compared with corn silage. This must be compensated for by increased consumption of either roughage or grain.

Grass silage usually contains a larger amount of protein than corn silage and, when used to replace corn silage, the protein content of the grain mixture may be lowered accordingly.

It must be remembered that grass silage is a variable product because of its moisture content, the crop from which it is made, and the manner in which it has been preserved.

SUMMARY AND CONCLUSIONS

Various combinations of molasses silage (grass, clover and alfalfa), corn silage and dry hay were fed to three groups of cows over a fifteen-week period. There was no significant difference in the production of the animals in the various groups, providing the total nutrient intake remained constant.

Molasses silage (grass, clover and alfalfa) was fed as the only roughage to eleven milking cows for twelve weeks. They maintained normal milk production and body weight. However, many of the animals were unable to consume the normal roughage nutrient intake on this diet. The decrease

in nutrient consumption was compensated for by an increase in the amount of grain.

In a ten-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found to be equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace corn silage in a normal ration of corn silage, mixed hay and grain.

TABLE 4

Chemical analysis of feeds used in experimental trials

Feed	Moisture	Protein	Ether extract	Crude fiber	Ash	Nitrogen free extract	Used in experiments
Corn silage	84.78	1.33	0.49	3.48	0.78	9.14	I
Corn silage	81.03	1.80	0.51	4.58	1.24	10.84	I
Corn silage	71.59	2.39	0.87	7.21	1.78	16.16	I
Molasses silage	80.25	1.36	0.53	6.27	1.01	10.58	I, II
Molasses grass silage	83.92	1.85	0.67	4.87	1.06	7.63	I, II
Molasses grass silage	74.52	2.76	1.14	7.68	1.58	12.32	I, II
Molasses grass silage	79.56	2.42	0.82	6.15	1.81	9.24	I, II
Mixed hay	5.41	8.74	2.02	28.87	5.96	49.00	I
Mixed hay	8.00	9.74	1.85	32.02	6.31	42.08	I
Mixed hay	6.21	10.91	2.30	28.52	6.81	45.25	I
Grain*	8.22	19.93	4.48	7.14	7.80	52.43	I, II
Corn silage	73.51	2.45	0.95	8.30	1.48	13.31	III
Molasses grass silage	65.41	6.33	1.18	11.08	2.90	13.10	III, IV
Phosphoric acid grass silage	70.21	2.71	0.80	11.24	2.00	13.04	III, IV
Grain*	9.77	19.37	5.40	8.94	6.24	50.28	III, IV
Mixed hay	8.27	15.34	1.76	35.02	7.39	32.22	IV

* Analyses according to Morrison (9) used in calculation.

In a twelve-week single reversal feeding trial, phosphoric acid silage (grass, clover and alfalfa) was found equal in feeding value for milk production and maintenance of body weight to molasses silage (grass, clover and alfalfa) when the grass silages were used to replace mixed hay in a normal ration of corn silage, mixed hay and grain.

TABLE 5

Digestion coefficients used (in per cent)

Feed	Protein	Ether extract	Crude fiber	Nitrogen-free extract	Experiment used	Reference
Corn silage	54.0	74.0	66.0	69.0	I, III	Morrison (9)
Grass silage	62.0	61.1	59.4	65.4	I, II, III, IV	Newlander *et al.* (10)
Mixed hay	59.0	58.0	51.0	69.0	I, IV	Morrison* (9)

* Used digestion coefficients for red clover hay all analyses.

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EFFECT OF PROTEOLYSIS ON LIPASE INDUCED RANCIDITY IN CHEDDAR CHEESE[1]

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Cheddar cheese made from milk to which a small amount of commercial lipase has been added will develop a flavor defect known as rancidity (1, 2). This effect is presumably due to lipolysis of butterfat and the consequent liberation of fatty acids, the lower members of which possess an unpleasant odor (3). Rancidity produced in this manner closely resembles the odor of rancid cheese which occurs occasionally but persistently under factory conditions. It has been suggested that milk lipase may be among the factors concerned in the development of rancidity under commercial conditions (4). Since lipases from various sources would be expected to possess many properties in common, the study of lipase induced rancidity is of interest in connection with the rancid cheese problem.

It has been known for some time that lipase is protein in nature (5), and that as such it is subject to inactivation by proteolysis (6). These observations have been extended by their application to the study of cheese flavor defects. In this investigation experimental evidence is submitted to show that rennet or pepsin or both inactivate, to some extent, added lipase in cheddar cheese and that the use of higher amounts of these proteolytic enzymes contributes to flavor improvement of such cheese.

EXPERIMENTAL

Milk was obtained from the Experimental Farm herd. Small vats of cheese were made using 240 pounds of milk. This gave two 10–12 lb. cheese to each vat. Cheese were made by an experienced maker according to standard procedures except as otherwise indicated below. They were stored at 60° F. until the first grading and then transferred to a second storage held at 48–50° F. The cheese were graded at intervals for flavor only by an experienced member of the Dominion Grading Staff.

It was first necessary to determine the amount of commercial lipase (Pfanstiehl) which when added to cheese milk would develop rancidity in 10 to 14 days. This was done by making a series of vats of cheese using varying amounts of lipase. It was found that 2.5–5.0 gms. of lipase per 1000 pounds of milk gave a satisfactory working level.

It was possible to show in preliminary experiments that higher than the

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usual amount of Hansen's rennet (*e.g.* 6 oz. per 1000 lb. milk) retarded to a certain extent the development of rancid flavor by lipase in cheese. The same effect was demonstrated for pepsin and for mixtures of rennet and pepsin. Pepsin from two sources was used; Armour's pepsin made for cheese making during the last war, and Merck's soluble pepsin. These are referred to later as pepsin A and pepsin M, respectively. Papain and trypsin (both Eimer and Amend products) were also tried but, owing to a bitter licorice-like flavor of the former and a high lipase content of the latter their use was discontinued.

The process of making cheddar cheese is not a rigid procedure. Such factors as the daily variation in the composition of milk, the starter culture, the rate of acid development, and the details of the manufacturing process are difficult to control exactly under experimental conditions. These make the interpretation of the results of grading rather difficult. In order to eliminate these variables the following method was adopted. In the morning all milk was pooled, brought to temperature and the starter culture added. When nearly sufficient lactic acid had developed, the milk was divided into two equal portions. To each vat was added an equal amount of lipase suspended in water. One vat was coagulated with a low amount of rennet while the other received a higher amount than normal and the make was then completed in the usual manner. Pepsin alone, and pepsin and rennet mixtures were also used instead of rennet. In this way each vat was as nearly identical as possible in every respect except in the amount of proteolytic enzymes used. Accordingly, one vat served as a comparison or reference standard for the other. Variation in the flavor score could then be attributed mainly to the effect of proteolytic enzymes on added lipase in the cheese.

The results are summarized in the accompanying table. Comparison vats (or mutual reference standards) as explained above, are indicated by A and B following the same number. The cheese of series A have in each case a lower content of proteolytic or coagulating enzymes than the corresponding members of the parallel B series. The amounts of rennet, pepsin and lipase are shown in the second column. The succeeding columns show the successive flavor scores on the respective cheese at indicated intervals.

An examination of the results shows that in general cheese of the B series have a higher flavor score than their corresponding mates of Series A. Since the cheese of the B series have also a higher content of proteolytic enzymes it is concluded, therefore, that proteolytic enzymes enhance cheese flavor by preventing the onset of rancidity due to added lipase.

DISCUSSION

In order to assess the value of the above experimental data accurately, careful consideration must be given to the various factors involved.

It will be noted that there are a few exceptions to the general statement

TABLE 1

Rennet, pepsin, lipase and flavor of experimental cheese

Cheese	Treatment per 1000 lbs. of milk			Flavor scores[1] of cheese during curing									
	Lipase	Rennet	Pepsin	Age in days	Score	Age in days	Score	Age in days	Score	Age in days	Score	Age in days	Score
	gm.	*oz.*	*gm.*										
1 A	5	2		9	38	16	38-	23	37-	86	37		
1 B	5	3¼		9	39-	16	38+	23	36	86	37		
2 A	2.5	2		11	38	18	38	32	36-	60	39-		
2 B	2.5	6		11	38+	18	39-	32	38-	60	39		
3 A	2.5	2		10	38-	17	37	31	37-	59	36		
3 B	2.5		86M	10	39-	17	38-	31	38-	59	36+		
4 A	5	3¼		11	38-	18	37	25	36	88	36		
4 B	5	3	30A	11	39	18	37-	25	36+	88	37-		
5 A	5	3¼		10	38	17	38	24	36	45	36	87	38-
5 B	5	3	43M	10	39-	17	38	24	38-	45	38	87	38+
6 A	2.5	3		9	38	16	38+	30	36	58	37+		
6 B	2.5	3	43M	9	39-	16	38	30	38	58	37		

[1] Flavor standards for grades of Canadian cheddar cheese.
First grade cheese—minimum score for flavor 39
Second " " " " " 37
Third " " " " " < 37
unless below third grade.

that the flavor scores on cheese in series B are higher than those of corresponding cheese in Series A. No explanation is offered for this discrepancy. In any event these exceptions do not invalidate the general conclusion.

Also, admittedly, some of the differences between the flavors on corresponding cheese are small. However, because of the system of comparison vats more significance can be attached to these results than could otherwise have been possible. The variations between any two different gradings is subject to some uncertainty because of the variation in the judgment of an individual grader at two periods remote from each other. However, differences in this direction are of secondary importance in this investigation.

It must also be borne in mind that if, on the one hand, larger amounts of lipase than the above established level are used, rancidity in cheese is developed to a greater intensity than that encountered commercially. The lipolysis of cheese fat is extensive during the initial stage and the beneficial effect of proteolytic enzymes cannot undo the damage already in existence. On the other hand, when smaller amounts of lipase are used in order to decrease the rate of lipolysis so that the remedial effect of rennet or pepsin or both can be established early, the differences between the flavor scores on

any two corresponding cheese are reduced. The interpretation of results is thus made more difficult. In obtaining the above data it was therefore necessary to select an arbitrary working level of moderate sensitivity.

The differences in the flavor score between corresponding cheese of the series A and B are quite consistent at the period of their first grading. This observation is of some importance because cheese are generally graded at 10–14 days in commercial practice. Although the results of subsequent gradings have also been included and the same general trend is shown, the maintenance of a high score flavor over prolonged periods of storage can be considered as a problem separate from that of the initial flavor scores.

Mention might be made of the types of flavor obtained in the above experimental cheese. It is possible to obtain typical butyric rancidity particularly in those cheese containing higher than the adopted lipase level. With lower amounts of lipase the flavors were often described as not straight rancid, unclean, dirty, etc. It has been pointed out in our preliminary communication (4) that these flavors resemble those known to occur commercially. Therefore, it has been suggested that lipase may not only be responsible for the rancid flavor but to some extent for certain less defined flavor defects.

Assuming that naturally present milk lipase does play a part in the development of cheese flavor (7) the above results suggest an additional role for the proteolytic enzymes in cheese. In addition to its coagulating function with its contingent effect on texture, and the proteolysis of casein or chemical ripening, rennet may play an active part in the inactivation of lipase, which when present in abnormal amounts in cheese milk would be expected to produce undesirable flavors.

Lipase induced rancidity has not been definitely identified with the rancid flavor occurring under factory conditions. Other factors may also be involved. The results of this investigation, however, are submitted because of a possible existing relationship. At the same time additional information on the proteolytic function of rennet has been obtained. Further study of lipase and its possible relation to rancidity in commercial cheddar cheese is being continued.

SUMMARY

Rancid and other less defined flavors have been reproduced in cheddar cheese by the addition of commercial lipase to cheese milk.

A higher flavor score was obtained in cheese to which lipase was added when higher amounts of pepsin or rennet or both were used in its manufacture than in corresponding cheese where smaller amounts of proteolytic enzymes were added.

An additional function has been attributed to the rennet enzymes.

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UTILIZATION AND EXCRETION OF ASCORBIC ACID BY THE DAIRY COW*

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The results of early studies (1, 2, 3) concerning the influence of diet upon the antiscorbutic potency of cow's milk, appeared to indicate that the vitamin C content of the milk paralleled that of the ration. During the same period, however, findings were reported (4) which led to the opposite conclusion, namely, that the ration received by cows had no influence on the antiscorbutic potency of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, further differences of opinion have arisen concerning the factors which influence the vitamin C content of cow's milk. As a result of recent work (5, 6, 7) the influence of breed and stage of lactation have been emphasized. Several workers (5, 8, 9) have attributed variations in the ascorbic acid content of milk to the season of the year. Other investigators (10, 11, 12, 13, 14) have concluded that the vitamin C content of cow's milk tends to be quite constant and is independent of the ration of the cow. Opposed to this view are those who still contend that the ascorbic acid content of the diet is a factor which cannot be ignored (6, 15).

A number of investigators have suggested that the cow is able to synthesize vitamin C (9, 13 ,16, 17, 18, 19). How or where this synthesis occurs is not understood, although one investigator (30) has claimed that the vitamin C of cow's milk is synthesized by the udder parenchyma and that the synthesis depends largely upon the condition of the udder.

The failure of numerous experiments involving standard dairy rations to establish clearly and conclusively the importance of the ascorbic acid content of the diet with regard to the amount of the vitamin in the milk, made it seem desirable to repeat previous work, but to alter the procedure by supplementing standard rations with known amounts of pure synthetic ascorbic acid. Moreover, it was desired to increase the significance of customary milk ascorbic acid analyses by determining, simultaneously, the amount of ascorbic acid in the blood and in 24-hour samples of urine.

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If, in the above proposed studies, it could be shown conclusively that the vitamin C content of milk is independent of the ration of the cow, it was hoped that some explanation could be obtained for the anomalous results just mentioned. It was desired, for example, to find new evidence for the synthesis of ascorbic acid in the cow. Further, it was hoped that some clue might be found regarding both the metabolic fate of ascorbic acid and the factors which influence its elimination from the body in the milk and in the urine.

EXPERIMENTAL

Methods and Apparatus. One or both of two chemical methods of estimation were used in all ascorbic acid analyses. These were the Tillmans titration method (20) employing standard 2,6-dichlorobenzenoneindophenol, and the newer Roe furfural method (21), which estimates total ascorbic acid (both reduced and reversibly oxidized ascorbic acid).

Milk samples were taken in a special apparatus which has been shown to preserve all of the ascorbic acid of the milk in the reduced form (22). The use of this apparatus permitted the quantitative determination of the vitamin by the convenient indophenol titration method.

Blood samples were taken in the conventional oxalated tubes under paraffin oil. For each test, 20 to 30 ml. of blood were taken from the jugular vein of the cow. As soon as the sample was obtained, the collection apparatus was placed inside a dark glass receptacle containing ice and water and was taken immediately to the laboratory for analysis. Essentially the Farmer and Abt macro-method (23) for plasma ascorbic acid was used for routine blood analysis.

Successful collections of the total 24-hour urinary excretion, free from fecal contamination, were made by employing a special rubber urine tube developed by Forbes and coworkers (24). The 18-liter carboys, which were used as receivers in the collection apparatus, were painted black to exclude light and were charged with enough glacial acetic acid to give a final concentration of about 5 per cent by volume. Addition of stick metaphosphoric acid to the acetic acid appeared to give no better results than when acetic acid was used alone; consequently, the addition of metaphosphoric acid was discontinued. In urinary ascorbic acid analyses, both the indophenol titration and the Roe furfural method were employed, with the exception of the earliest work on one of the cows which was done before the furfural technique had been reported. This double analysis seemed desirable in view of the lack of unanimity among various workers concerning the specificity of present methods for the determination of ascorbic acid in urine. Moreover, it appeared probable that some of the vitamin would be unavoidably oxidized to dehydroascorbic acid during the collection of a 24-hour sample. Such has since been shown to be true with human urine (25). The furfural method

is particularly valuable in this case because it determines both dehydro- and reduced ascorbic acids.

It was recognized at the outset that it would be desirable to eliminate from the projected work the effect of breed differences by limiting the experiments to one breed of dairy cow. Holstein cows were eventually chosen, largely because they were available for experimental purposes at the time this work was started.

Effect of a Standard Ration. In order to have values which might later be used to compare with those obtained during administration of ascorbic acid, each cow was maintained on a standard ration for an interval of time during which analyses were made to determine the concentrations of ascorbic acid in the blood, the milk, and the urine. The averages of the values obtained during typical five-day test periods are given in table 1. Five-day

TABLE 1

*Summary of results obtained when Holstein cows were fed a standard ration and when they were fed a standard ration supplemented with ascorbic acid**

	S. R.	C. S.	G. C.	D.
Mg. ascorbic acid per ml. milk	0.019	0.021	0.021	0.020
Mg. ascorbic acid per 100 ml. plasma	0.53	0.58	0.50	0.48
Mg. ascorbic acid per day in urine—				
Indophenol titration	45.7	52.7	54.1	26.8
Furfural method	910.5	833.1	643.5	

S. R.—Standard ration.

C. S.—Standard ration supplemented with 50 to 100 grams of ascorbic acid mixed with corn silage.

G. C.—Standard ration supplemented with 50 grams of ascorbic acid in gelatin capsules.

D.—Standard ration supplemented with 50 grams of ascorbic acid administered by drenching.

* The values given in Table 1 represent the averages obtained in experiments employing from two to four cows, except in the case of the ascorbic acid administered by drenching, where the values are from an experiment with a single cow.

sampling periods were considered representative for any diet, in view of the fact that all cows received a particular ration for several days, or even weeks, prior to the actual collection of samples.

Effect of Ascorbic Acid Added to Corn Silage. In choosing methods for administration of massive amounts of ascorbic acid, it was decided to administer some of the vitamin mixed with a small amount (3–5 pounds) of corn silage. It was found that 50–200 grams (1,000,000–4,000,000 International Units) of ascorbic acid placed in such a mixture were consumed by a cow in a period of about 10 minutes. In contrast, ascorbic acid mixed with grain was eaten very slowly, if at all.

Experimental periods were designed to include a two-day interval during which the cow received the standard ration alone, followed by a three-day period during which she received the standard ration supplemented each day with a certain amount of crystalline ascorbic acid mixed with corn silage,

which was succeeded by a post-administrative period of two days during which the animal again received only the standard ration. The same procedure was used in subsequent experiments during which the ascorbic acid was administered in gelatin capsules and by drenching. The data from such experimental periods are summarized in table 1.

From a comparison of values in table 1, it is apparent that supplements of ascorbic acid administered by three different methods had no significant influence upon the concentrations of ascorbic acid in the blood, milk, or urine of the cows used in these experiments.

Effect of Ascorbic Acid Injected Intravenously. Rasmussen and coworkers (26) reported that intravenous injection of ascorbic acid resulted in a marked temporary rise in the vitamin C content of the milk of the ewe and cow. It was desired to repeat this work and to enlarge upon it by making analyses of blood and urine as well as of milk. Therefore, experiments were performed during which ascorbic acid was injected into the blood stream via the jugular vein. 24 grams of crystalline ascorbic acid, dissolved in sterile water, were given in this manner on each of two or three successive days. The effect of these ascorbic acid injections was studied with three cows with corresponding results. Typical values are given for one of the cows in table 2.

TABLE 2

Concentration of ascorbic acid in the blood, the milk, and in the urine of a Holstein cow during feeding of a standard ration supplemented with intravenous injections of ascorbic acid

Day	Mg. ascorbic acid per 100 ml. plasma	Mg. ascorbic acid per ml. milk	Mg. urinary ascorbic acid per day	
			Indophenol titration	Furfural method
1	0.58	0.021	24.3	611.1
2	0.58	0.020	34.6	241.5
3*	2.74**	0.020 0.019 0.022	6922.9	13311.8
4*	4.42**	0.024 0.028 0.028	9870.0	13632.6
5*	4.86**	0.030 0.029 0.027	14715.2	18194.0
6	0.86	0.028 0.025 0.022	8813.6	20923.8
7		0.021	161.2	604.7
8		0.020	117.8	982.8

* 24 grams of ascorbic acid dissolved in 100 ml. of sterile water were injected into the jugular vein. The multiple values for milk ascorbic acid represent each of the three daily milking periods.

** Blood analyses were made on samples taken 1½ hours after injection of ascorbic acid.

The intravenous injection of ascorbic acid produced unmistakable increases in the concentration of that vitamin in the milk and urine as well as in the blood. A very large portion of the injected ascorbic acid which could be accounted for appeared in the urine. In the case of one cow, this amounted to over ninety per cent of the total ascorbic acid injected. These results correspond closely to those recently reported (31) for experiments in which ascorbic acid was injected into goats.

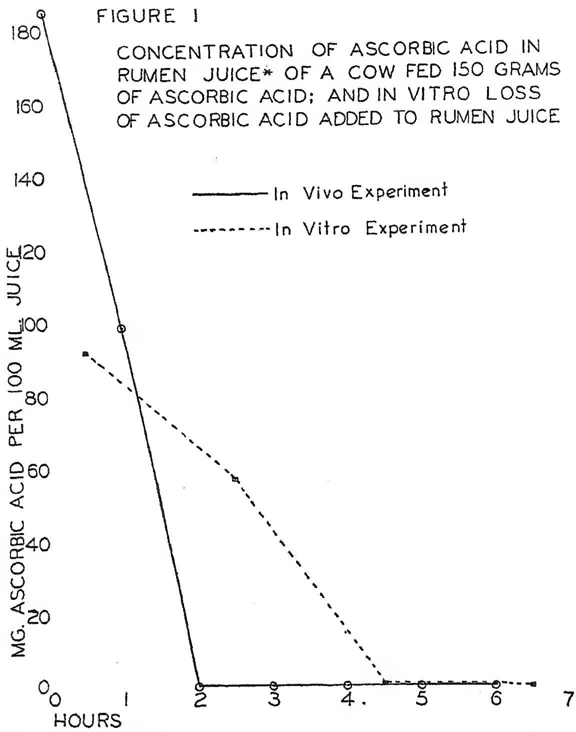
Effect of Ascorbic Acid Injected Subcutaneously. The experiments described thus far seemed to indicate that ascorbic acid administered by non-injection methods never reached the blood stream in significant amounts. Further, data from the intravenous injection experiments showed that the concentration of ascorbic acid in the milk was definitely increased if large amounts of the vitamin reached the blood stream. In order to further substantiate these findings, it was desired to administer some ascorbic acid by a method which would not place the vitamin directly in the blood stream but which would insure the ultimate arrival of large amounts in the circulation. Such a purpose was accomplished by setting up an experiment in which ascorbic acid was injected subcutaneously in regions around the cow's forelegs.

The effect of the subcutaneous injections of ascorbic acid closely resembled that of the intravenous injections. The influence of the subcutaneous injections upon blood, milk, and urinary ascorbic acid values was more gradual and somewhat less pronounced than in the case of the intravenous injections. During the course of three successive daily injections of 24 grams of ascorbic acid, the ascorbic acid titer of the milk was raised from 0.020 to 0.027 mg. per ml. and that of the urine from 600 to a peak of 15,000 mg. per day.

Studies with a Rumen Fistula. At this point in the experiments it appeared to be almost certain that the failure of massive amounts of ingested ascorbic acid to influence the concentration of the vitamin in the milk or to significantly alter its concentration in the blood and urine, could be attributed to a destruction of this substance in the rumen. To investigate this possibility, a rumen fistula was created in one of the cows. With this permanent opening leading directly into the largest compartment of the cow's stomach, it was possible to study the fate of ingested ascorbic acid by removal and analysis of partially digested food at intervals after feeding.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after feeding supplements of ascorbic acid and after insertion of the vitamin directly into the rumen. During such periods, the average concentrations of ascorbic acid in the blood plasma, in the milk, and in the urine were respectively 0.42 mg. per 100 ml., 0.019 mg. per ml., and 1260.5 mg. per day, values which do not differ significantly from those obtained during feeding of a standard ration alone.

The disappearance of ascorbic acid from the rumen contents during an experiment in which 150 grams of ascorbic acid were fed is shown graphically in figure 1. A preliminary report on this experiment has been given elsewhere (32).



* By the term ''rumen juice'' is meant the liquid portion of rumen contents.

In order to demonstrate that volume changes were not responsible for the pronounced decrease in the ascorbic acid concentration of the rumen contents as shown above, an experiment was performed in the laboratory with a controlled volume of rumen contents. 1000 ml. of rumen contents, from which the coarse particles of feed had been removed by straining through cheesecloth, were placed in a dark-glass, wide-mouth bottle. To this mixture, which had a pH of 6.50, was added 1000 mg. of crystalline ascorbic acid. After thoroughly mixing the contents, the bottle was loosely stoppered and placed in a water bath maintained at a temperature of 39° to 42° C. At intervals, samples were removed for analysis.

Figure 1 shows that the ascorbic acid disappeared just as it had in the *in vivo* experiments with the exception that the *in vitro* decrease proceeded at a more gradual rate. This slower rate of destruction of ascorbic acid in the *in vitro* experiment as compared to the *in vivo* experiments seems readily

explained by the absence of the continual stirring and the circulation of gases so characteristic of the rumen.

DISCUSSION OF RESULTS

State of Ascorbic Acid in Urine. As soon as the furfural method was applied to the urine analyses it became apparent that the results obtained ranged from one and a half to thirty times as high as those obtained by the indophenol titration. During injections of ascorbic acid, however, this discrepancy narrowed to a point where, in some cases, the results obtained by the two methods almost coincided. These facts, together with the knowledge that the indophenol and furfural methods of analysis checked well when applied to 24-hour samples of rat urine collected under similar conditions (33), suggested that important amounts of the ascorbic acid excreted daily by a cow on a standard ration were excreted in the form of dehydroascorbic acid. In order to test this hypothesis, samples of urine were collected as they were excreted and analyzed at once. Table 3 gives the results obtained

TABLE 3

Reduced and oxidized ascorbic acid in cow's urine immediately following urination

Cow No.	Reduced ascorbic acid Mg. per liter Indophenol titration	Total ascorbic acid Mg. per liter Furfural method
1	20.9	17.0
2	33.2	25.4
3	19.7	18.2
4	10.1	10.3
5	27.7	25.1
6	27.6	25.4
7	53.1	48.1
8	40.1	43.3
9	21.5	19.0
10	31.0	29.6

from Holstein, Jersey, Brown Swiss, and Ayrshire cows. These findings seem to indicate that all the ascorbic acid excreted in the urine of the dairy cow is in the reduced form. The greater values obtained in eight out of ten cases by the indophenol method are readily explained by the recognized tendency to go beyond the endpoint, which is obscured to an extent proportional to the concentration of the urine pigments. The greater values obtained by the furfural method for 24-hour samples of urine must indicate, therefore, that variable amounts of the excreted ascorbic acid are oxidized to dehydroascorbic acid during the interval between excretion and analysis, or that the urine contains appreciable amounts of non-ascorbic acid furfural precursors capable of forming a derivative with 2,4-dinitrophenylhydrazine. Tests for the latter interfering substances were made repeatedly and in no case showed the presence of concentrations sufficiently large to interfere with

the method. Consequently, it may be concluded that while all the ascorbic acid excreted in the urine is originally in the reduced form, some of it is oxidized to dehydroascorbic acid under the conditions involved in the collection of a 24-hour sample of urine.

Destruction of Ascorbic Acid in the Rumen. Several explanations might be given for the rapid and pronounced disappearance of ascorbic acid from the rumen after oral administration of massive amounts of the vitamin. It might be argued that the large amounts observed in the first two or three samples represented stages in the incomplete mixing of the ascorbic acid with rumen contents. Changes in the concentration of the rumen ascorbic acid could also be attributed to an intake of water by the animal, or by a passage of a portion of the rumen contents to other chambers of the stomach.

Another interpretation of the findings is that the very soluble vitamin was rapidly absorbed. This has been suggested by Riddell and Whitnah (27). These workers studied the fate of vitamin C in the rumen contents of a cow with a rumen fistula and in a steer at slaughter. In each case the rumen contents were found to contain less than one-tenth the vitamin C of green rye ingested twelve hours earlier. These workers explained the rapid disappearance of ascorbic acid from the rumen by suggesting that ascorbic acid was rapidly absorbed. The basis for this suggestion was the observation of a temporary doubling of the vitamin C content of the blood within 12 hours and a fivefold increase in the ascorbic acid content of the urine within 60 hours after green feed was first supplied.

The most plausible explanation, however, and the one demanded by our experimental data, is that a rapid and pronounced destruction of ingested ascorbic acid occurs in the rumen. Thus, while the other factors which have been mentioned undoubtedly have some influence on the results observed, it is hardly likely that they account for changes of the magnitude indicated in figure 1.

The incomplete mixing theory is especially inadequate. When a cow is eating, the rumen contracts about three times per minute and each contraction causes a flow of liquid throughout the rumen and its solid contents. While figure 1 indicates that the first sample was removed at zero time, it was actually taken about five minutes after the animal had eaten the last of the ascorbic acid treated silage or 15 minutes after she had started to eat. This means that the ascorbic acid had been subjected to 15 to 45 contractions of the rumen. Further, when the cow was slaughtered, it was found that the rumen contained 38 liters of liquid or semi-liquid material. If 150 grams of ascorbic acid were thoroughly distributed throughout this liquid, there should be a concentration equivalent to about 400 mg. per 100 ml. of juice. Figure 1 indicates that the concentration of ascorbic acid in the juice, as shown by analysis of the first sample removed, was about 185 mg. per 100 ml. of juice. From these facts, it would appear that fairly complete mixing had occurred, accompanied by extensive destruction of the vitamin.

Observations of this and other dairy cows show that they drink water infrequently. It is difficult to say how much and how often liquid material passes permanently from the rumen into other compartments of the stomach. Ewing and Wright (28), working with steers, found that the average rate of passage of food residues through the rumen and reticulum was 61 hours. In any event, credence in the volume-change explanation for the disappearance of ascorbic acid from the rumen is seriously discounted by the results of *in vitro* constant-volume experiments such as shown in figure 1.

Indirect evidence that ingested ascorbic acid is largely destroyed in the rumen rather than being rapidly absorbed is given in the blood, milk, and urine ascorbic acid values, which, with the possible exception of the urine, show no response to the feeding of 50 grams or more of ascorbic acid. Why there is always a small amount of ascorbic acid in the rumen, as there appears to be, is difficult to explain. Possibly the release of this vitamin from solid feed fragments proceeds gradually and at a rate slightly higher than the rate of destruction. We hope to continue the study of the factors responsible for the destructive effects described.

In the light of the demonstrated destruction of ascorbic acid in the rumen, it becomes clear why various methods of oral administration of the vitamin failed to produce a response in the milk or other body fluids. If the ascorbic acid administered in gelatin capsules had been able to survive rumen conditions and reach other compartments of the stomach, it seems likely that other results might have been obtained, for the ascorbic acid content of the milk of non-ruminating animals, *e.g.*, guinea pigs (26) and humans (8, 29), has been shown to be influenced by diet. In work with the rumen fistula, however, it was found that gelatin capsules were dissolved and the ascorbic acid was released after 10–15 minutes in the rumen.

SUMMARY

1. Special equipment was employed which permitted the complete collection from dairy cows of 24-hour samples of urine free from any fecal contamination. It was found impractical, if not impossible, to preserve all the ascorbic acid in such urine samples in the reduced form.

2. By the simultaneous application of the indophenol titration and the furfural method of analysis to freshly excreted samples of urine, it was possible to show that ascorbic acid is excreted in cow's urine in the reduced form.

3. Analysis of over 50 samples of blood obtained from four Holstein cows showed that the ascorbic acid content of the plasma ranged from 0.43 to 0.62 mg. per 100 ml. when the cows received standard dairy rations.

4. Ascorbic acid was administered to Holstein cows (a) mixed with a small amount of corn silage, (b) in gelatin capsules, (c) in aqueous solution, (d) intravenous injection, and (e) by subcutaneous injection. Administration of as high as 100 grams (2,000,000 International Units) of ascorbic acid per day for three days by a non-injection method failed to increase the ascor-

bic acid concentration of the milk or blood and had only a slight effect on the concentration of the vitamin in the urine. It was only by the injection methods that a significant increase in the ascorbic acid concentrations of the blood, milk, and urine could be demonstrated. The greatest increase in milk ascorbic acid concentration during experiments in which 24 grams of ascorbic acid were injected intravenously on each of three successive days, was from 20 mg. per liter to 30 mg. per liter.

5. A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed as much as 150 grams (3,000,000 International Units) of synthetic ascorbic acid at one time; similar amounts were also placed directly in the rumen through the fistula opening. A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals. Ascorbic acid added to rumen contents *in vitro* and stored in a dark-glass, stoppered receptacle at 39°–42° C. disappeared at much the same rate as that of the *in vivo* experiments. In making analyses, both the indophenol titration and the Roe furfural method were employed.

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STORAGE OF DAIRY BULL SPERMATOZOA*

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An appreciation of the limitations and possibilities of storing dairy bull semen and retaining its fertility is necessary in order to define the practical limits of artificial insemination with shipped or stored semen. While various diluents have been proposed and different storage temperatures have been suggested, there still remain many unexplained factors concerning the successful storage of dairy bull semen. One difficulty in semen storage research has been the lack of an accurate criterion of fertility. Since the time of survival with vigorous motility of bull spermatozoa stored undiluted at 40° F. has been shown to be correlated with the fertility of bull semen (14), this property of the semen may be used in evaluating storage methods more accurately than simple determination of time of survival.

Refrigeration has been recognized for some time as the most effective method of preserving bull semen (15). Spermatozoa normally live only a short while at body temperature, and room temperature (70° F.) has likewise been found too high for successful semen storage (17). The highest temperature at which spermatozoa may be stored successfully has been given as 50° F. (4), and longer viability was secured with reduction of the storage temperature to 35° to 40° F. (4, 7, 8, 17). It has been emphasized that the change of temperature of the semen must be gradual or irreversible immotility will result from temperature shock (2, 6).

Many diluents of various composition have been used with bull semen, but their main use has been in increasing the volume (15, 4). The addition of nutrient substances to these diluents did not increase the survival of spermatozoa in them (1). The buffering action provided by some diluents has increased the motility of spermatozoa (8). A diluent using egg yolk plus a buffer has been reported as causing a marked increase in survival and fertility of stored bull semen (11).

The secretions of the accessory sex glands were shown to be harmful to survival of spermatozoa and were entirely unsuitable as diluting fluid for bull semen (5, 10, 11). With horse and boar semen the removal of the sperm fluid and concentration of the spermatozoa has resulted in greater survival in storage (16, 9). Experiments along this line with bull semen were not successful in obtaining greater survival in storage.

Early investigators excluded air from the semen by a layer of paraffin

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oil (15). Recent work has shown that such procedure is not necessary and that spermatozoa live longer with free access to oxygen (12, 13).

Although there are records of cows being settled with semen eight days old, the practical limits of storage of bull semen have been given as only 12 to 30 hours (3).

This paper presents the results of studies to find the most practical method of storing bull semen so as to preserve its fertility. Investigations were made as to the desirable temperature for storage, the benefits to be derived from using various diluents, the possibility of removing the accessory sex gland secretions to improve survival, and the actual effectiveness of stored semen as measured by its fertility when used in artificial insemination.

EXPERIMENTAL AND RESULTS

The semen used in these experiments was collected by use of the artificial vagina during the winter and spring of 1940 from ten bulls in the University of Missouri dairy herd.

Motility determinations were made at 250 × magnification of a drop of semen placed on a microscope slide in a stage incubator at 100° F. Motility was rated from 0 to 5. No motility was 0, and 5 represented the very best grade of motility. Weak, oscillatory motion with less than 40 to 50 per cent of the spermatozoa moving was rated 1. Progressive degrees of motility from 1 to 5 were rated 2, 3, and 4. Unless otherwise stated, all storage was made in an ordinary electric refrigerator operating at about 40° F. As soon after collection as possible, nearly always less than 30 minutes, the semen was put in small sterile glass vials, stoppered with freshly paraffined corks, wrapped in paper towelling, covered with rubber cots, and placed in a tray of water at 40° F. in the refrigerator. Cooling was thus made gradual and was accomplished within an hour after collection. A part of each sample was stored untreated at 40° F. as control for part of the same sample which was used in the experiment.

Effect of temperature on viability of semen. The storage temperature was observed to have a marked effect upon the rate of loss of motility. Thirty-seven samples representing ten different sires were stored at 70° to 75° F., while duplicate samples of the same semen were stored at 40° F. The averages of the motility ratings of these samples at various storage periods are presented in figure 1. At the end of six hours the semen stored at 70° F. showed slightly more vigorous motility than did that stored at 40° F. After 16 hours storage, motility in samples stored at 40° F. was much superior to that in samples stored at 70° to 75° F. Sixteen of the 37 samples stored at 70° to 75° F. were non-motile at 24 hours, and at 40 hours all except one were dead, while semen from the same samples stored at 40° F. still had good, vigorous motility.

A few samples of semen were stored at 31° to 32° F. Motility ratings of semen stored at this temperature fell more rapidly than motility in semen stored at 40° F., but the spermatozoa were still viable by the time motility rating in semen stored at 40° F. was reduced to 1. Thus, although motility was reduced, the time of survival was not greatly reduced.

Effect of diluting solutions on sperm viability. Samples of semen were divided to compare the effect upon viability in storage when diluted with

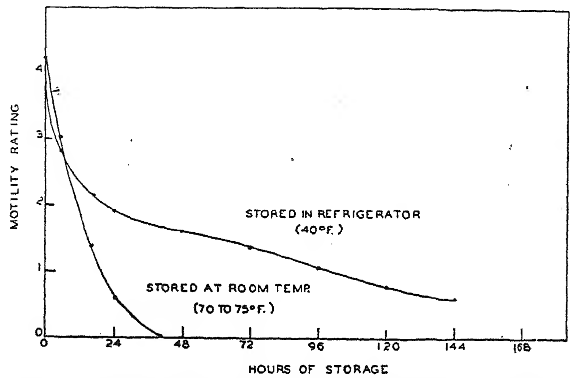

FIG. 1. Average maintenance of motility in 37 samples of semen stored at 40° F. and 70° to 75° F.

various diluents. The dilution rate used was three parts of diluent to 1 part of semen. The first diluents tried were 3 and 5 per cent glucose solutions and Milovanov's (10) S-G-C-2 dilutor. All of these were definitely harmful to the spermatozoa as measured by their effect upon motility maintenance. Motility was more vigorous in the undiluted semen in every case although the time of survival did not differ greatly between the diluted and undiluted semen.

The egg-yolk buffer (E-Y-B) dilutor which was proposed by Phillips *et al.* (11) was used in 18 samples of semen from eight different bulls. The comparison of the part of these samples diluted with E-Y-B dilutor with the part stored undiluted is shown in figure 2. On the average the use of this diluent was definitely favorable to sperm motility up to the fifth day of storage.

Thereafter, the semen which was diluted perished rapidly while that which was not diluted remained motile to a low degree. The effect of the E-Y-B dilutor varied greatly as to the semen of different bulls in which it was used. Semen from two bulls which was quite viscous and highly concentrated was greatly benefited by dilution with E-Y-B dilutor. Semen of these bulls was normally of very good initial motility, but the undiluted semen became very viscous in storage and motility declined rapidly. Semen of the other bulls which was more nearly normal in characteristics was

benefited very little if any by the use of this dilutor. In only one case, however, was the motility rating in the E-Y-B diluted semen less than that in the undiluted semen until the fifth day of storage. When the E-Y-B dilutor was added to undiluted semen which had become of low motility in storage, no reactivating or stimulating effect was secured even though a part of the same sample which had been stored diluted with E-Y-B dilutor initially had good motility at the time. In storage periods exceeding five days, the undiluted fractions of the semen survived longest.

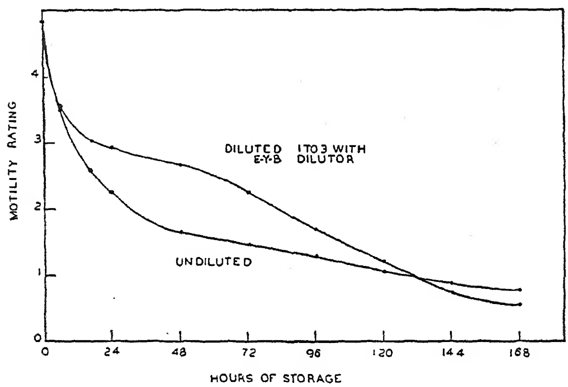

FIG. 2. Average motility ratings of semen stored undiluted and of semen from the same samples diluted 1 to 3 with egg-yolk buffer dilutor.

Effect of removal and replacement of the sperm fluid upon motility and survival of spermatozoa. Since the secretions of the accessory sex glands had been shown to be harmful to survival of the spermatozoa (5, 10, 11), the effect of removal of part of these secretions was studied. Separation was accomplished by centrifuging the fresh semen at 1300 RPM for 15 minutes. The relative centrifugal force was approximately 375 grams. The supernatant fluid which was clear and practically devoid of spermatozoa was removed by use of a pipette.

Spermatozoa stored in the concentrated state could not be reactivated with physiological saline or five per cent glucose solutions after 48 hours of storage, while the same semen untreated showed very vigorous motility at 48 hours. Replacing the sperm fluid with S-G-C-2 dilutor or three per cent glucose solution immediately after centrifuging proved of little value for maintaining motility. Although a low motility rating was maintained for three to five days in semen so treated, the motility in the untreated semen was very much better and was maintained long after the treated semen had died.

Replacing the sperm fluid with four volumes of E-Y-B dilutor immediately after centrifuging was decidedly beneficial to the spermatozoa for the

first four days of storage. The average results of such treatment of 14 samples of semen from six bulls compared with motility ratings of the same samples untreated are presented in figure 3. Here again it was noticed that very good motility was secured from spermatozoa stored in E-Y-B dilutor for four days. On the fifth day of storage, however, motility was as good in the untreated as in the treated semen; and thereafter the treated semen died rapidly while a low motility rating was maintained for some time in the natural semen. In six of the 14 samples, however, survival time was as long or longer in the treated as in the natural semen.

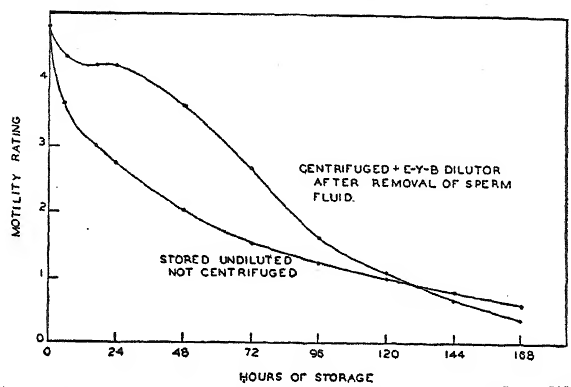

FIG. 3. Comparison of motility maintenance in semen stored undiluted and semen stored after replacing sperm fluid with egg-yolk buffer dilutor.

Since egg yolk contains practically no glucose, and since the sperm fluid containing glucose had been removed, glucose was added to three centrifuged E-Y-B diluted samples of semen with hopes of prolonging the life of the centrifuged spermatozoa. Semen so treated reacted practically the same as identical semen treated in the same way but without glucose. Hence glucose as such apparently was not the limiting factor in causing the sudden, early death of the centrifuged spermatozoa.

Insemination with stored semen. The stored semen was used for inseminations at every opportunity as it seemed that this practice should give the best test of maintenance of fertility. During the winter and spring of 1940, thirty inseminations were made with stored semen of which 15 resulted in conception. The complete record of these inseminations, including the age of the semen when used and its motility characteristics is given in table 1. These results show that the failure of certain samples of semen to produce conception was correlated with quality of the semen, irrespective of age. There was considerable variation even in samples from the same bull as to the time they could be stored successfully. The record indicates that the undiluted semen was as effective as either that diluted with E-Y-B dilutor or that centrifuged and diluted with E-Y-B dilutor. There is also indication

TABLE 1

Record of inseminations using stored semen

Bull No.	Age of semen when used (hrs.)	Motility rating at time of insemination	Maintenance of 2 motility in semen (hrs.)	Maintenance of 2 motility after use (hrs.)	Remarks
		Inseminations which resulted in conception			
47	74	3	96	22	E-Y-B diluted
47	198	2	198	0	E-Y-B diluted; shipped 400 mi.
48	4	3	....	......	Shipped 118 mi.
48	68	1	24	0	Double insemination
48	11	3	24 +	13 +	Shipped 150 mi.
48	10	3	....	......	Shipped 150 mi.
49	48	5	72	24	Shipped 40 mi.
49	72	3	96	24	
54	44	4	144	100	
54	24	4	72	48	
40	115	3	120	5	
50	24	2	24	0	
50	4	4	24	20	Shipped 40 mi.; cow with vaginitis
50	72	2	120	48	
50	20	3	48	28	Double insemination-shy breeder
Ave.	52.5	3.0	81.6	25.5	
		Insemination which failed to impregnate			
47	20	4	....	......	
47	125	2	196	71	E-Y-B diluted, shipped 400 mi.
55*	55	1	24	0	E-Y-B diluted
55*	48	4	48	0	E-Y-B diluted
49	24	4	96	72	Cow was shy breeder
49	72	4	96	24	Same sample as above
49	120	3	144	24	
49	56	4	48	0	E-Y-B diluted and centrifuged
49	16	4	72	56	E-Y-B diluted and centrifuged
53*	20	1	6	0	Double insemination
53*	24	1	6	0	
50	96	2	96	0	
50	4	4	24	20	Heifer with vaginitis
50	4	4	24	20	Semen shipped 40 mi.
50	24	2	24	0	
57	5	1	0	0	Semen shipped 150 mi.; bull's first service in 2 weeks.
Ave.	47.2	2.7	62.9	19.1	

* Bulls of low fertility.

that semen of poor motility at the time of insemination was inffective, although there were exceptions. One cow was settled with semen showing a motility rating of only 1. Some samples of semen were used on two cows, one of which conceived and the other did not, so evidently the genital mechanism of the cow is quite important in affecting the use of stored semen. The

oldest semen which resulted in conception (also the oldest tried) was stored 198 hours. A live calf has now been dropped as a result of this insemination. The next oldest semen was stored 115 hours. Three samples stored over 90 hours failed to produce conception. The ratio of two services per conception for use of all the stored semen, considering all types of cows and bulls included in the study, is not excessive. There is indication that a fair conception rate should result from use of good quality semen from fertile bulls which has been stored 48 to 72 hours, provided that it shows good motility at the time of insemination.

DISCUSSION

In handling or preparing semen for storage, much care is necessary. The full initial power and energy of the spermatozoa must be preserved insofar as possible. Temperature shock should be avoided by changing the temperature gradually. The storage temperature should be reached promptly after collection, however, and it should be low. Ordinary household refrigerator temperature of 40° F. has been found satisfactory. This study has clearly shown that room temperature (70° F.) is too high for storage of bull semen. Temperature of melting ice (32° F.) was too low for best motility maintenance, although semen can be kept alive for long periods at that temperature. It may be that the cooling and warming around 32° F. are too rapid, or that temperatures so low may have a deleterious effect on the spermatozoa.

The use of diluents to aid in preserving fertility of spermatozoa was not necessary. Although the egg-yolk buffer dilutor increased the vigor of motility for the first few days of storage, it did not prolong the life of good quality semen; and in the few actual inseminations of cows in which it was tried, it was not superior to the undiluted semen in fertility. In view of this fact, the main use of diluents seems to be still one of increasing volume. In cases where an increase in volume is desired, however, the egg-yolk buffer dilutor should be used in preference to others herein reported as it was the only one which gave a favorable effect to spermatozoa motility. The short beneficial action of the egg-yolk buffer does not seem to be by way of nutrition of the spermatozoa for they died quicker in it than they did in good quality undiluted semen stored at 40° F.

Increasing the concentration of bull spermatozoa was not conducive to sperm survival. Removal of the accessory sex gland fluids by centrifuging did increase motility, however, when a favorable diluent was used for replacement. Since storage of the concentrated spermatozoa was not successful, it appears that bull spermatozoa require some medium, probably for the elimination of waste products, for best survival. The special treatment necessary to centrifuge and dilute bull semen may have valuable application

in the shipment of semen over long distances where it will be used within 48 to 72 hours. Further practical work is necessary to prove this.

CONCLUSIONS

1. Ordinary household refrigerator temperature of 40° F. was entirely satisfactory for storage of bull semen.

2. Room temperature or near freezing temperature was undesirable for storage of bull spermatozoa, although the effect of the latter was not markedly injurious.

3. Glucose solutions and Milovanov's S–G–C–2 dilutor were of no value in increasing the length of survival time in stored bull semen.

4. Egg-yolk buffer dilutor (Phillips, 11) was beneficial to motility of bull spermatozoa for the first 100 hours of storage. Semen varied in its reaction to dilution with this diluent.

5. Very vigorous motility was obtained for the first 100 hours of storage by removing the sperm fluid from the spermatozoa and diluting with egg-yolk buffer dilutor.

6. Good quality semen survived longer when stored *undiluted* than when diluted with any of the diluents tried.

7. Good quality semen which has been stored undiluted at 40° F. can be used for insemination with a reasonable degree of fertility for storage periods up to two to three days. Conception with dairy bull semen stored at longer intervals is possible, as demonstrated in this study, but the practicability of using such semen is questionable in view of the low ratio of pregnancies resulting.

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QUANTITATIVE DETERMINATION OF ALPHA AND BETA LACTOSE IN DRIED MILK AND DRIED WHEY

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INTRODUCTION

The physical properties of many milk products, particularly dried milk and whey, are greatly influenced by lactose. Therefore, it is important to know whether the lactose is in the crystalline state and, if so, whether crystallized as the alpha or the beta form and the extent to which crystallization has progressed.

The presence of alpha hydrate and beta crystals can be demonstrated qualitatively by using the seeding tests described by Sharp (2). Troy and Sharp (3) described briefly a method for determining the relative amounts of alpha and beta lactose. They did not give details because the method had not been studied sufficiently at that time. The present article gives the details of procedure and some of the supporting evidence on the points of technique involved. The basis of this method is to obtain quickly a clarified solution of the product, polarize the solution at once, allow the solution to stand until the forms of lactose reach equilibrium, and finally polarize again. The relative amounts of alpha and beta lactose are calculated from the change in rotation; the amount of total anhydrous lactose is calculated from the final rotation.

REAGENTS AND APPARATUS

Alcoholic mercuric chloride. Dissolve 264 grams of mercuric chloride in 1000 ml. of 95 per cent ethyl alcohol, hold overnight and filter. Each determination requires 10 ml.

Norrit suspension. Suspend 120 grams of norrit in 900 ml. of water, add enough N HCl to give a pH of 4–5 and then make up to 1 liter. Each determination requires 5 ml. of the freshly agitated suspension.

Previous to preparing the suspension 125 grams of norrit should be refluxed 10 hours with 1 liter of 1 per cent hydrochloric acid and washed by refluxing repeatedly with distilled water. Acid extraction is not necessary with some samples of norrit; also the alkalinity of some samples is not sufficient to require adjusting of the pH to 4–5 if heavily buffered solutions are analyzed.

Citric acid solution. A solution containing 0.2 to 0.3 per cent citric acid mono-hydrate is prepared. This solution is needed to obtain clear filtrates from badly heated or brown whey; otherwise water is used. Each analysis of dried whey requires 45 ml.

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Oxalic acid dihydrate. Crystalline solid, about 0.02 to 0.04 gram used for each determination; required for all dried milk.

Oxalic acid solution. A solution containing 0.9 gm. oxalic acid dihydrate per liter is prepared. All dried milk samples are dissolved in 45 ml. of this solution.

Zinc chloride. Crystalline solid about 0.1–0.2 gram used for each determination on dried whey if required.

Apparatus. Balance, porcelain mortar and pestle, 100 ml. volumetric flask, erlenmeyer flask, funnel, watch glasses, fluted crepe filter paper, polariscope, stop-watch, 4 decimeter water-jacketed polariscope tube and intense light (589 mμ).

METHOD

Clarification procedure. Weigh 2.500 grams of the dried milk or whey and transfer to a dry porcelain mortar (the sample taken should contain not less than 1 gram nor more than 2.5 grams of lactose). Dry grind the sample for approximately a minute in the mortar, add 2.0 ml. of distilled water at 17° C. to a whey, or 10 ml. oxalic acid solution at 17° C. to a milk. Start a stop watch and wet grind to produce a smooth thin paste which then grind vigorously. Add more solvent and continue to grind until about 30 ml. have been added. Pour the mixture into a 100 ml. volumetric flask, into which has previously been introduced 5 ml. of norrit suspension. Rinse the mortar with additional 3 to 4 ml. portions of solvent until a total amount, 45 ml. at 17° C., has been used. (If the final filtrate obtained from a dried whey is not clear the citric acid solution should be substituted for water up to this point.) Complete the rinsing with 15–25 ml. of water at 25° C. (wash bottle) and thus transfer the entire sample with the lactose completely dissolved and extracted, to the volumetric flask. Swirl the contents. Swirling is advised to reduce foaming.

Add to the volumetric flask 10 ml. of the ethyl alcoholic solution of mercuric chloride. Rotate and swirl the contents of the flask 0.5–1.0 minute in order to mix thoroughly. Make up to the 100 ml. mark with distilled water at 25° C. When alcohol and water are mixed a slight rise in temperature results. For this reason, the temperature of the water used in extracting and making up the solution should be adjusted so that the final temperature after mixing and diluting to the mark will be approximately 25° C. The elapsed time at this stage should be 2 to 3 minutes. Shake and mix the contents of the volumetric flask for 30 seconds and pour the entire contents of the flask at once onto a fluted crepe filter paper. (This filter should not be folded too tightly or broken paper fibers will be delivered with the filtrate.) Return the filtrate to the filter until delivered clear. Then filter directly into a dry, water-jacketed polariscope tube maintained at a temperature of 25° C. Collect the remainder of the filtrate in a dry erlenmeyer flask. Keep the filter covered to minimize evaporation.

Polariscopic readings. As soon as the polariscope tube is filled a reading is taken, and additional readings are taken at minute intervals until a total of 11 has been made. Unless the temperature of the solution is 25° C. at the start the first few readings will be in error. The first reading of the polariscope is usually obtained within 5 minutes after starting the stop watch. Occasionally the time will be somewhat longer, if the solution filters slowly.

The 11 polariscopic readings are plotted against the time, as indicated by the readings of the stop watch. The best line is drawn through these points, and it is extrapolated to zero time—that is, the time of adding the water and starting of the stop watch. The readings of the polariscope extrapolated to zero time give the initial rotation, I, of the solution. The final rotation is obtained after the solution has stood at least 8 hours. It is often most convenient to add a drop of toluene and allow the solutions to stand overnight at room temperature (25° C.), in flasks. Ten readings of the final rotation are made, and the average, called F, is used in the equations given below. If a precipitate forms on standing overnight, filter the solution. Whey filtrates in which colloidal material is suspended can usually be cleared by addition of 0.1–0.2 gram of solid zinc chloride. It may be necessary to heat the solution in a water bath for a few minutes until flocculation occurs. The solution is filtered through a fine-grained filter paper.

Milk filtrates generally become turbid and cannot be clarified satisfactorily by means of zinc chloride. These are treated with 0.02–0.04 gram of solid oxalic acid dihydrate and filtered through a fine-grained filter after precipitation is complete.

Calculations. The relative amounts of anhydrous alpha lactose or of beta lactose are obtained by substituting the initial (I) and the final (F) rotations in one of the two equations (7) or (8), given below:

$$\%\text{ anhydrous alpha lactose} = \left(\frac{I}{F} - 0.635\right) 101.1 \qquad (7)$$

$$\%\text{ beta lactose} = \left(1.624 - \frac{I}{F}\right) 101.1 \qquad (8)$$

If information is required only as to the relative amounts of alpha and beta lactose present, it is not necessary to weigh the sample taken for the determination; but by taking an accurately weighed sample of 2.500 grams, the amount of lactose in the product can easily be determined from the final reading by multiplying the final reading by 18.15 if a 4 decimeter tube is used. If a saccharimeter instead of a polarimeter is used, the value must be changed to its equivalent reading on the saccharimeter scale.

SIGNIFICANCE OF DETAILS

The principle on which this method is based is simple and obvious. The difficulty arises in obtaining quickly a water-clear extract in which the

mutarotation of the sugar has not been accelerated by the protein precipitants. Most agents used for clarifying protein solutions are not satisfactory because they accelerate mutarotation, and we are thus limited to those precipitations which can be carried out in a neutral (pH 4–6) solution. A great number of protein precipitates were investigated both singly and in combination. An alcoholic solution of mercuric chloride was found to be the most satisfactory.

Solution of the sample. If the lactose is not dissolved before adding the alcohol, the rate of solution is generally so retarded by the alcohol as to delay the determination of the initial rotation; or some of the lactose may actually be filtered out if sufficient time is not allowed for solution. Relatively large lactose crystals are present in some samples, and solution is not sufficiently rapid unless the sample is dry ground in the mortar before the water is added.

Adding the water to the sample in the mortar permits the immediate disintegration of the lumps which form in many samples and enables the extraction to be made quickly and completely.

The extraction in the mortar is made at about 17° C. so that the heat liberated when the alcoholic mercuric chloride is added later will bring the temperature to 25° C. Unless the temperature is 25° C. at the time of the first polariscopic reading, the first few values will be in error; for the differences in refractive index produced by the difference in temperature of portions of liquid in the polariscope tube will make matching of the fields difficult.

The amount of water used at the various stages has been carefully worked out to permit the complete solution of the lactose and transfer of the sample to the 100 ml. volumetric flask, and to obtain a temperature of 25° C. in the final mixture.

Alcoholic mercuric chloride precipitation. Alcohol was used because it is a good solvent for mercuric chloride and because it also aids in the protein precipitation and prevents adsorption of lactose by the norrit. Norrit was required to remove the yellow color produced by the riboflavin and the brown color of the milk and whey produced by heat or aging. The amount of norrit recommended sufficed to decolorize all but abnormally brown (overheated or very old) samples. The slight residual yellow color of some filtrates obtained from such samples did not seriously reduce visibility in the polariscope.

Turbidity in a filtrate, slight enough to be almost imperceptible when viewed in a flask, becomes disturbing when viewed through a 4 dm. polariscope tube. A light of high intensity is recommended for fine work. We used a monochromator adjusted with calibrated quartz plates.

Varying concentrations of mercuric chloride in the alcohol, varying amounts of alcohol and varying amounts of norrit were tried and a combina-

tion of the three was worked out which gave the best results. If too much alcohol is used the solubility of the lactose is affected and also its specific rotation; yet sufficient alcohol should be used to aid in the precipitation and prevent adsorption of lactose by the norrit.

Norrit. The norrit is added in the form of a water suspension because the air contained in the dry material causes marked foaming, whereas in the suspension the air has been eliminated from the surface of the norrit.

A trace of lactose seems to adsorb on the norrit even in the presence of alcohol. Irregular behavior was occasionally observed when unextracted norrit was used. No correction is applied for the volume of the norrit (0.4 ml.) or of the protein precipitate. This is because the corrections are small, and because the amount of lactose adsorbed and the effect of the alcoholic mercuric chloride on the rotation of the lactose approximately compensate for the concentrating effect of the volume displaced by the solid phase.

To retard mutarotation, it is advisable to adjust the acidity of the norrit suspension to pH 4–5 with hydrochloric acid, and such adjustment is necessary when pure unbuffered lactose solutions are analyzed. In the analysis of dried milk and dried whey, if the highest accuracy is not desired, the extraction with hydrochloric acid and the adjustment of the pH of the norrit suspension can be dispensed with. The unextracted norrit suspensions tested had pH values of 8 to 9. The buffer value and pH of the dried milk are sufficient to reduce the pH and prevent marked acceleration of mutarotation by the alkaline norrit.

Clouding of filtrates and development of cloudiness: Most dried milks and some dried wheys, if dissolved in water, give turbid filtrates after precipitation with alcoholic mercuric chloride. A number of substances were added in the attempt to obtain clear filtrates and at the same time not interfere with the determination.

The best results were obtained with dried milks by dissolving them in the oxalic acid solution recommended. This served to delay the appearance of turbidity. Its mode of action is not clear. If the turbidity is due to a colloidal calcium compound, the oxalic acid might either delay its formation, or partially precipitate it.

Some dark samples of dried whey must be extracted with the citric acid solution as a solvent in place of water in order to obtain clear filtrates for determining the initial rotation.

Precipitation occurs in many whey filtrates and most milk filtrates during equilibration.

The turbidity of whey filtrates is usually removed by filtering through a fine paper; milk filtrates generally remain turbid. Turbidity in whey filtrates which is not removed by simple filtration can be precipitated by treatment with solid zinc chloride (0.1 – 0.2 gm.); in milk filtrates by treat-

ment with solid oxalic acid dihydrate (0.04 gm.). Warming accelerates flocculation. The flocculated material is removed with a fine filter.

Adding alkalies to accelerate final rotation. Many investigators recommend the addition of alkalies so that the final rotation can be obtained almost immediately after obtaining the initial rotation. Sodium carbonate, sodium bicarbonate and ammonium hydroxide have been recommended. This procedure, unless tested with the specific product, may lead to errors. If too much alkali is added the optical rotation of the sugar is affected. If not enough alkali is added, the mutarotation may not be accelerated as expected. Correct final rotations are obtained by acceleration with alkali only when the solution is adjusted to the proper pH. The addition of the same amount of alkali to different samples will not give the same pH because of the different buffer values, particularly in the case of dried wheys. Moreover, the addition of alkali precipitates mercuric oxide in the filtrates here employed. For these reasons it is much safer to allow the solution to reach equilibrium by standing rather than to attempt to accelerate mutarotation by alkali.

Derivation of equations. Several values are given in the literature for the specific rotations of the two forms of lactose and of their equilibrium mixture for the sodium D lines at 20° C. (1). Direct determinations at 25° C. are not reported. The specific rotations obtained in this laboratory at 25° C. with light of 589 mμ using products of high purity are as follows: anhydrous alpha lactose (weighed as the hydrate), + 89.5° beta lactose, + 35.0°; equilibrium mixture at concentrations between 10 and 250 gm. anhydrous lactose per liter, + 55.1°. All three constants apply to aqueous solutions.

Suppose A_o per cent of the lactose in a sample were in the alpha form, then $100-A_o$ represents the per cent in the beta form. The initial specific rotation, I_s, of a solution obtained from this sample would be given by

$$89.5A_o + 35\ (100-A_o) = 100\ I_s \tag{1}$$

where the constants are the specific rotations of alpha and beta lactose respectively.

On equilibration at 25° C. the solution would assume a final specific rotation, F_s, and in this solution A_∞ per cent of the lactose would be in the alpha form, $100-A_\infty$ per cent in the beta form. For this solution

$$89.5A_\infty + 35\ (100-A_\infty) = 100\ F_s \tag{2}$$

Dividing (1) by (2) and collecting terms gives,

$$\frac{I_s}{F_s} = \frac{54.5A_o + 3500}{54.5A_\infty + 3500} \tag{3}$$

In the equilibrium solution at 25° C., however, we have:

$$89.5A_\infty + 35\ (100-A_\infty) = 100 \times 55.1 \tag{4}$$

where 55.1 is the specific rotation of this solution. Solving (4) for A_∞ we obtain 36.9 and substituting this value in (3) and solving for A_o:

$$A_o = \left(\frac{I_s}{F_s} - 0.635\right) 101.1 = \%\ \text{alpha} \tag{5}$$

and

$$(1\text{–}A_o) = \left(1.624 - \frac{I_s}{F_s}\right) 101.1 = \%\ \text{beta} \tag{6}$$

Since the observed initial and final rotations, I and F, are made with the same solution in the same polariscope tube they are proportional to I_s and F_s and can be substituted in (5) and (6) to give (7) and (8).

$$A_o = \left(\frac{I}{F} - 0.635\right) 101.1 = \%\ \text{alpha} \tag{7}$$

$$(1\text{–}A_o) = \left(1.624 - \frac{I}{F}\right) 101.1 = \%\ \text{beta} \tag{8}$$

These percentages, of course, refer to total amount of anhydrous lactose in the sample as 100.

Accuracy of the method. Tables 1 and 2 show results obtained when the method was applied to the pure alpha and beta forms of lactose respectively. Inspection of table 1 shows that filtration and such possible impurities in the alcohol as acetaldehyde and acetic acid do not influence the results. Mercuric chloride and alcohol, individually and combined, so affect the rotation as to give apparent percentages of total lactose slightly below 100, and apparent percentages of alpha lactose slightly above 100.

Norrit adsorbs appreciable amounts of lactose from aqueous solution but only a very limited amount from solutions containing alcohol.

The effects of norrit, alcohol, mercuric chloride and combinations of these reagents on beta lactose solutions are shown in table 2. The results are similar to those obtained with solutions of alpha lactose. In the presence of alcoholic $Hg\ Cl_2$ the apparent percentage of total lactose is a little low, but the percentage of beta lactose is as nearly correct as the errors in the method would justify. Adsorption of beta lactose on the carbon is shown and the adsorption is also inhibited by alcohol.

Preferential adsorption of alpha lactose on norrit. Not only does norrit adsorb appreciable amounts of lactose from an aqueous solution but it adsorbs the alpha modification preferentially. This can be demonstrated clearly when appreciable amounts of both the alpha and beta forms are present in the solution, and when enough norrit is added to adsorb about one half of the lactose present. A dry mixture containing 40 per cent by weight of alpha lactose (calculated on the anhydrous basis) and 60 per cent of beta lactose was prepared. Aliquots containing 0.74 gram anhydrous lactose were dissolved and made up to a final volume of 100 ml., using variations in procedure which would demonstrate adsorption of lactose by norrit and elution by alcohol. The results are presented in table 3.

Experiments A, B, C and D show no appreciable effect of filtration and only a slight effect of 20 per cent alcohol on the ratio and rotations of the

TABLE 1

Analysis of alpha lactose hydrate, sugar weighed on anhydrous basis

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	8.96	5.511	1.626	100.2	−0.2	100.0
	8.96	5.508	1.627	100.3	−0.3	100.0
	8.93	5.504	1.622	99.8	0.2	99.9
	8.94	5.508	1.623	99.9	0.1	100.0
Water and lactose alone, filtered	8.96	5.488	1.633	100.9	−0.9	99.6
	8.93	5.482	1.629	100.5	−0.5	99.5
	8.95	5.510	1.624	100.0	0.0	100.0
	8.92	5.514	1.618	99.4	0.6	100.1
In 4.5% alcohol	8.95	5.492	1.630	100.6	−0.6	99.7
In 9 % alcohol	8.94	5.479	1.632	100.8	−0.8	99.4
In 18 % alcohol	8.87	5.449	1.628	100.9	−0.9	98.9
In 4.5% alcohol, filtered	8.96	5.489	1.632	100.8	−0.8	99.6
In 9 % alcohol, filtered	8.94	5.479	1.632	100.8	−0.8	99.4
In 18 % alcohol, filtered	8.81	5.437	1.620	99.6	0.4	99.4
In 1.2% $HgCl_2$	8.98	5.480	1.639	101.5	−1.5	99.5
In 2.3% $HgCl_2$	8.94	5.472	1.634	101.0	−1.0	99.3
In 4.5% $HgCl_2$	8.94	5.474	1.633	100.9	−0.9	99.4
In 1.2% $HgCl_2$, filtered	8.98	5.464	1.643	101.9	−1.9	99.2
In 2.3% $HgCl_2$, filtered	8.97	5.475	1.638	101.4	−1.4	99.4
In 4.5% $HgCl_2$, filtered	8.91	5.466	1.630	100.6	−0.6	99.2

TABLE 1—(*Continued*)

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
+ 10 ml. 26.4% alcoholic $HgCl_2$	8.85	5.457	1.622	99.8	0.2	99.0
	8.88	5.445	1.631	100.7	−0.7	98.8
+ 10 ml. 26.4% alcoholic $HgCl_2$, filtered	8.90	5.473	1.626	100.2	−0.2	99.3
	8.91	5.484	1.625	100.1	−0.1	99.5
	8.92	5.479	1.628	100.4	−0.4	99.4
+ 20 ml. 26.4% alcoholic $HgCl_2$, filtered	8.85	5.431	1.630	100.6	−0.6	98.6
+ 10 ml. 26.4% alcoholic $HgCl_2$ containing .75% acetaldehyde	8.92	5.466	1.632	100.8	−0.8	99.2
+ 10 ml. 26.4% alcoholic $HgCl_2$ containing .25% acetic acid	8.95	5.467	1.637	101.3	−1.3	99.2
+ .60 gm. norrit in suspension	8.52	5.236	1.627	100.3	−0.3	95.0
	8.56	5.257	1.628	100.4	−0.4	95.4
+ 10 ml. 26.4% alcoholic $HgCl_2$ + .60 gm. norrit in suspension	8.85	5.450	1.624	100.0	0.0	98.9
	8.85	5.449	1.624	100.0	0.0	98.9
	8.82	5.451	1.618	99.4	0.6	98.9
	8.85	5.451	1.624	100.0	0.0	98.9

TABLE 2

Analysis of beta lactose

	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total lactose in product %
	Initial I	Final F	$\frac{I}{F}$	As Alpha %	As Beta %	
Water and lactose alone	3.51	5.514	.637	0.2	99.8	100.1
	3.48	5.492	.634	−0.1	100.1	99.7
Water and lactose alone, filtered	3.50	5.529	.633	−0.2	100.2	100.4
	3.52	5.527	.637	0.2	99.8	100.3
+10 ml. 26.4% alcoholic $HgCl_2$	3.49	5.489	.636	0.1	99.9	99.6
	3.46	5.476	.632	−0.3	100.3	99.4
+10 ml. 26.4% alcoholic $HgCl_2$ filtered	3.47	5.476	.634	−0.1	100.1	99.4
	3.48	5.475	.636	0.1	99.9	99.4
+.60 gm. norrit in suspension	3.36	5.249	.640	0.5	99.5	95.3
	3.33	5.257	.633	−0.2	100.2	95.4
+.60 gm. norrit in suspension +10 ml. 26.4% alcoholic $HgCl_2$	3.54	5.467	.648	1.3	98.7	99.2
	3.51	5.485	.640	0.5	99.5	99.6
	3.47	5.494	.632	−0.3	100.3	99.7
	3.47	5.478	.633	−0.2	100.2	99.4

forms of lactose. In experiment E about half of the lactose was adsorbed on the norrit and the proportion of the two forms remaining unadsorbed was altered from that of the original solution, as shown by the decrease in the ratio $\frac{I}{F}$. If the forms were adsorbed in proportion to their concentration in the original solution, then the filtrate would be relatively richer in alpha lactose, but actually it is poorer. Furthermore, experiment F showed that when the adsorbed sugar was eluted from the norrit by alcohol the eluate was richer in alpha as shown by the increase in the ratio $\frac{I}{F}$. This demonstrates the preferential adsorption of the alpha form by norrit. Data obtained in a series of other experiments not recorded here can be explained on this basis.

In experiment G lactose in solution was added to a mixture of norrit suspension and alcohol. Practically no adsorption took place and the ratio of the two forms in the filtrate remained unchanged. The previous adsorption of lactose by the norrit does not alter the ratio of alpha to beta in the filtrate when the norrit surface is later freed of lactose by the addition of alcohol as the last step in preparing the mixture. This was demonstrated in experiment H. Experiments A, B, C, D, G, and H show good agreement with the calculated values, for the rotations and also the proportions of alpha and beta lactose in the mixture.

Recovery of lactose added to dried milk and dried whey. Table 4 shows the percentage recovery of specific forms of lactose when added to dried milk

TABLE 3

Adsorption of lactose by norrit, preferential adsorption of the alpha form and elution with alcohol

The suspension of extracted norrit added, contained 2.2 grams. In each experiment of 0.74 gram of anhydrous lactose was made up to 100 ml. and the solutions were polarized at 25° C. in a 4 decimeter tube.

Expt.	Preparation of the solutions	Rotations			Lactose present as		Total lactose in sample %
		I	F	$\frac{I}{F}$	Alpha %	Beta %	
A	Dissolved in water.	1.679 1.680	1.620 1.616	1.036 1.040	40.5 40.9	59.5 59.1	99.3 99.1
B	Dissolved in water, filtered.	1.672 1.675	1.617 1.623	1.034 1.032	40.3 40.1	59.7 59.9	99.1 99.5
C	Dissolved in H_2O; sol'n. added to alcohol + H_2O; contained 20% alcohol after diluting to mark.	1.648 1.643	1.610 1.612	1.024 1.019	39.3 38.8	60.7 61.2	98.7 99.8
D	Dissolved in H_2O; sol'n. added to alcohol + H_2O; contained 20% alcohol after diluting to mark; filtered.	1.655 1.635	1.610 1.593	1.028 1.026	39.7 39.5	60.3 60.5	98.7 97.7
E	Dissolved in H_2O; sol'n. added to norrit, brought to volume, centrifuged, decanted, filtered.	0.842 0.854	0.858 0.862	0.981 0.991	35.0 36.0	65.0 64.0	52.6 52.8
F	Packed norrit from above extracted with 20% alcohol.	0.760 0.829	0.715 0.767	1.063 1.081	43.3 45.1	56.7 54.9	43.8 47.0
G	Aqueous sol'n. of sugar added to norrit + alcohol; sol'n. contained 20% alcohol diluting to mark.	1.662 1.674	1.606 1.623	1.035 1.031	40.4 40.0	59.6 60.0	98.5 99.5
H	Aqueous sol'n. of sugar added to norrit for adsorption; sugar eluted by adding alcohol before diluting to mark; filtrate contained 20% alcohol.	1.658 1.642	1.612 1.599	1.029 1.027	39.8 39.6	60.2 60.4	98.8 98.0
I	Mixture as made by weighing, calculated.	1.681	1.631	1.031	40.0	60.0	100.0

TABLE 4

Recovery of added lactose

Lactose added: 1 gm. pure anhydrous Alpha lactose (weighed as hydrate) or 1 gm. pure anhydrous Beta lactose.

Sample analyzed	Lactose added	Rotations, 4 dm. tube 25° C.			Amount of lactose		Total lactose in product %	Gm. anhydrous lactose in filtrate			Per cent added lactose recovered		
		I	F	$\frac{I}{F}$	As Alpha %	As Beta %		Total	Alpha	Beta	Total	Alpha	Beta
Skimmilk 40 (glass)	None	2.75	2.609	1.054	42.4	57.6	47.4	1.1837	0.5019	0.6818	....	....	....
	Alpha	6.37	4.847	1.314	68.6	31.4	....	2.1991	1.5086	0.6905	101.5	100.7	....
	Beta	4.21	4.874	0.864	23.2	76.8	....	2.2114	0.5130	1.6984	102.8	....	101.7
Whey 1 (Alpha)	None	5.96	3.818	1.561	93.6	6.4	69.3	1.7322	1.6213	0.1109	....	....	....
	Alpha	9.57	6.028	1.588	96.3	3.7	....	2.7349	2.6337	0.1012	100.3	101.2	....
	Beta	7.46	6.084	1.226	59.8	40.2	....	2.7603	1.6507	1.1096	102.8	....	99.9
Whey 2 (Beta)	None	2.57	3.595	0.715	8.1	91.9	65.2	1.6311	0.1321	1.4990	....	....	....
	Alpha	6.19	5.827	1.062	43.2	56.8	....	2.6437	1.1421	1.5016	101.3	101.0	....
	Beta	4.03	5.822	0.692	5.8	94.2	....	2.6414	0.1532	2.4882	101.0	....	98.9
Whey 67 (Alpha)	None	5.51	3.470	1.588	96.3	3.7	63.0	1.5743	1.5161	0.0582	....	....	....
	Alpha	9.01	5.647	1.596	97.2	2.8	....	2.5621	2.4904	0.0717	98.8	97.4	....
	Beta	6.96	5.667	1.228	60.0	40.0	....	2.5711	1.5427	1.0284	99.7	....	97.0

TABLE 5

Amount of alpha and beta lactose in selected samples of dried whey and milk

Sample No. and nature	Rotations 4 dm. tube, 25° C.			Amount of lactose		Total anhydrous lactose in product %
	I	F	$\frac{I}{F}$	% As Alpha	As Beta %	
51. Whey, beta type	2.58 2.55	3.45 3.47	0.748 0.735	11.4 10.1	88.6 89.9	62.6 63.0
53. Whey, beta type	3.31 3.27	3.84 3.96	0.862 0.826	22.9 19.3	77.1 80.7	69.7 71.9
71. Whey, beta type	3.00 2.97	3.64 3.67	0.824 0.809	20.1 17.6	79.9 82.4	66.1 66.7
58. Whey, beta type	3.37 3.35	3.70 3.76	0.911 0.891	27.9 25.9	72.1 74.1	67.2 68.2
64. Whey, beta type	3.19 3.22	3.63 3.65	0.879 0.882	24.7 25.0	75.3 75.0	65.9 66.2
F. Whey, beta type	3.07 3.13	3.73 3.74	0.823 0.837	19.0 20.4	81.0 79.6	67.7 67.9
82. Modified whey	2.27 2.26	2.20 2.20	1.032 1.027	40.1 39.6	59.9 60.4	39.9 39.9
24. Whey, alpha type, pan condensed	5.40 5.47	3.56 3.58	1.517 1.528	89.2 90.3	10.8 9.7	64.6 65.0
40S. Whey, alpha type, pan condensed	6.13	3.88	1.580	95.5	4.5	70.4
41S. Whey, alpha type, pan condensed	5.81	3.74	1.553	92.8	7.2	67.9
67. Whey, alpha type, pan condensed (old analysis)	5.62 5.60	3.63 3.62	1.548 1.547	92.3 92.2	7.7 7.8	65.9 65.7
65. Whey, alpha type, spray dried	5.09 5.13	3.48 3.51	1.463 1.462	83.7 83.6	16.3 16.4	63.2 63.7
72. Whey, alpha type, spray dried	5.18 5.24	3.43 3.48	1.510 1.506	88.5 88.1	11.5 11.9	62.3 63.2
10. Skimmilk, roll dried	2.70	2.56	1.055	42.5	57.5	46.5
11. Skimmilk, roll dried	2.12	2.02	1.050	42.0	58.0	....
M20. Skimmilk, roll dried	2.92	2.75	1.062	43.2	56.8	49.9
M40. Skimmilk, roll dried	2.76	2.60	1.062	43.2	56.8	47.2
M60. Skimmilk, roll dried	2.69	2.55	1.055	42.5	57.5	46.3
M80. Skimmilk, roll dried	2.51	2.43	1.033	40.2	59.8	44.1

or whey samples. The recovery of total lactose of each individual form is as good as might be expected. The data in table 4 show an increase in experimental error, when the method is applied to dried milk or whey—as compared to the errors seen in tables 1 and 2 where pure sugars were analyzed. Several additional potential sources of error are introduced in analyzing dried milk and whey.

Alpha and beta lactose in samples of dried milk and whey. Table 5 presents analyses of various typical dried products and shows the wide variations encountered in the relative amounts of alpha and beta lactose, ranging from about 90 per cent in the beta to 95 per cent in the alpha form. The amount of total lactose in the products also showed considerable variation.

SUMMARY

1. A method is described for the determination of the amounts of alpha and beta lactose in dried milk and whey, and also of the total amount of anhydrous lactose in these products.

2. A clear, colorless extract of the product is obtained quickly, using an alcoholic solution of $Hg\ Cl_2$ as protein precipitant, and norrit as a decolorizing agent. The filtrate is polarized as soon as possible and again after standing. The relative amounts of alpha and beta lactose are computed from the change in optical rotation. Total lactose is calculated from the final (equilibrium) rotation and sample weight.

3. Alpha and beta lactose gave satisfactory results when analyzed by this method, as did also dried milk and whey, alone and with additions of alpha and beta lactose.

4. The composition of the lactose in dried whey was shown to range from 90 per cent beta to 95 per cent alpha, on the anhydrous basis.

5. Norrit adsorbs lactose from aqueous solution, the alpha form being adsorbed preferentially. Lactose is eluted from the norrit by 10 to 20 per cent alcohol.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. III. EFFECT OF VARIATIONS IN TIME AND TEMPERATURE OF INCUBATION AND OF STORAGE ON ACTIVITY OF CULTURES*

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The variation permissible in methods of preparation and storage of cultures of lactic acid bacteria without causing reduction in their activity remains a practical problem to the dairy manufacturer. In a previous paper Elliker and Frazier (1) reported the influence of incubation temperature on heat resistance of certain starter organisms for Swiss cheese; in their experiments, incubation periods common in actual practice were employed. The present study was undertaken to ascertain how much variation in time and temperature of incubation was permissible without loss in activity of the starter organisms. The effect of these same variations on heat resistance of cultures will be discussed in the following paper of this series.

A definite relationship between the titratable acidity of starter cultures grown at a constant temperature and the quality of Swiss cheese was observed by Frazier and associates (3). The most effective milk starter cultures of *Lactobacillus helveticus*, Strain 39a, had titratable acidities of 1.00 to 1.09 per cent (as lactic acid) after growth at 37.5° to 39° C. for 12 hours. In experiments with cultures of *Streptococcus thermophilus*, Strain C–3, best results were noted when milk cultures showed titratable acidities of 0.70 to 0.75 per cent after 12 hours at 37° C. Thus the preparation of starter cultures requires controlled temperatures and periods of incubation for production of the desired rate of lactic acid fermentation in the cheese in the press. Information bearing on the methods or procedures of preparing active starter cultures should be helpful not only to the makers of Swiss cheese but also to all who handle active cultures of thermoduric lactic acid bacteria.

EXPERIMENTAL

Pure cultures of *Lactobacillus helveticus*, Strain 39aW, and *Streptococcus thermophilus*, Strain C–3, were grown at constant temperatures in freshly autoclaved ten per cent reconstituted skimmilk. After seven to nine consecutive transfers at a given temperature, the activity of each mother culture, as evidenced by amount of growth and fermentation, was de-

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termined. For bulk culture, an inoculum of 0.25 per cent, from a mixture of equal parts of mother culture and a two per cent solution of sodium citrate, was transferred into 400 ml. of fresh skimmilk medium. Bulk cultures were incubated for 12 hours, and usually at the same temperature as that previously used for the mother culture. The amount of growth in bulk cultures was determined by direct microscopic count of living cells, according to the method of Frazier and Boyer (2). Acidity of bulk cultures was measured by titration, and pH was calculated from the potentials shown by the quinhydrone electrode. Direct counts were made also of mother cultures after incubation; in some studies, the acidities of mother cultures were used for comparison. Representative experiments are summarized briefly.

Influence of incubation time of mother cultures grown at 37° C. on the activity of 12-hour bulk cultures. In a series of several experiments the cultures were incubated at 37° C. and transferred every 12, 24, 36, 48 and 168 hours for seven to nine successive transfers. The recommended period for incubation of Swiss cheese starters has been 12 to 14 hours at 37° C., so that their maturity could be judged by the acidity produced in that time. A comparison of activity of 12-hour bulk cultures prepared from mother cultures incubated at 37° for periods longer than those commonly employed is made in table 1; arithmetic averages of several experiments are given.

TABLE 1

Differences in activity of bulk cultures after 12 hours at 37° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37° C. and transferred every 12, 24, 36, 48 or 168 hours*

Mother culture		Activity of bulk culture		
Incubation time	Direct count	Direct count	Titratable acidity	pH
hours	*millions/ml.*	*millions/ml.*	*per cent*	
		L. helveticus		
12	1,013	1,294	1.05	4.10
24	1,041	1,249	1.04	4.18
36	796	703	0.77	4.42
48	714	464	0.63	4.70
168	519	2	0.26	5.90
		Str. thermophilus		
12	1,032	1,079	0.82	4.41
24	1,017	1,363	0.81	4.46
36	797	597	0.73	4.51
48	732	380	0.63	4.67
168	410	19	0.27	5.88

* Seven to nine successive transfers.

Cultures of *L. helveticus* or *Str. thermophilus* after numerous transfers at 37° C. every 12 or 24 hours were similar in growth and fermentation in 12-

hour bulk cultures. Periods of incubation greater than 24 hours, however, resulted in a decrease in the numbers of cells in both mother cultures and 12-hour bulk cultures, while the acidities developed in bulk cultures decreased progressively as the age of mother culture increased.

Influence of incubation time of mother cultures grown at 40°, 42°, or 45° C. on the activity of 12-hour bulk cultures. Since relatively short periods (12 to 24 hours) of incubation of mother cultures grown at 37° C. resulted in no marked change in the activity of 12-hour bulk cultures, higher temperatures of incubation for similar periods were employed. It might be expected that the incubation of mother cultures at temperatures above 37° and for periods greater than 24 hours would seriously decrease acid production in 12-hour bulk culture. The results of preliminary experiments substantiated this belief.

TABLE 2

Differences in activity of bulk cultures after 12 hours at 40°, 42°, or 45° C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 40°, 42°, or 45° C. and transferred every 12 and 24 hours

Mother culture		Activity of bulk culture			Incubation temperature of all cultures
Incubation time	Direct count	Direct count	Titratable acidity	pH	
hours	*millions/ml.*	*millions/ml.*	*per cent*		*°C.*
			L. helveticus		
12	880	1,070	1.08	4.01	40
24	871	967	0.99	4.21	40
12	1,579	1,309	0.97	4.10	42
24	864	1,028	0.93	4.19	42
12	....	....	0.46	5.13	45
24	....	....	0.68	4.58	45
			Str. thermophilus		
12	739	828	0.70	4.63	40
24	634	733	0.70	4.64	40
12	1,348	1,211	0.76	4.35	42
24	1,444	923	0.74	4.38	42
12	....	....	0.70	4.66	45
24	....	....	0.67	4.65	45

The effect of cultivation of mother cultures at 40°, 42°, and 45° C. for successive 12- and 24-hour periods upon the activity of 12-hour bulk cultures is shown in table 2. Mother cultures of *L. helveticus* grown at 40° or 42° C. for 12 hours were slightly more active in 12-hour bulk culture than the 24-hour mother cultures; however, incubation at 45° gave irregular data, apparently due to the close approach of the incubation temperature to the maximum temperature of growth of this organism. A comparison of the data on the bulk cultures prepared from 12- and 24-hour mother cultures of *Str. thermophilus* provides no conclusive evidence that the 12-hour mother

cultures at a given temperature of incubation are superior in fermentation or growth to the 24-hour mother cultures carried at the same temperature.

Differences in mother cultures as shown by the activity of bulk cultures in the initial phases of the growth cycle. Although differences in acidity and total cell counts could not be demonstrated in 12-hour bulk cultures, the possibility that differences in growth and fermentation existed during the first few hours after inoculation was investigated. A comparison of bulk cultures, made 2.5 to 3 hours after inoculation, appears in table 3.

TABLE 3

Comparison of amount of growth and fermentation of bulk cultures shortly after inoculation with mother cultures which had been transferred at different temperatures every 12 and 24 hours

Mother culture		Activity of bulk culture				
Incubation		Incubation		Direct count of inoculum	Direct count after incubation	Drop in pH
Time	Temp.	Temp.	Time			
hours	*°C.*	*°C.*	*hours*	*millions/ml.*	*millions/ml.*	
L. helveticus						
12	37	37	2.5	1.82	8.42	0.06
24	37	37	2.5	1.60	19.1	0.07
12	37	42	2.5	1.33	16.6	0.13
24	37	42	2.5	1.08	12.7	0.11
12	40	40	3.0	2.02	31.9	0.22
24	40	40	3.0	2.21	15.9	0.04
12	42	42	3.0	....	....	0.07
24	42	42	3.0	....	....	0.02
Str. thermophilus						
12	37	37	2.5	.810	109.	0.26
24	37	37	2.5	.916	77.0	0.20
12	40	40	2.5	.410	45.0	0.34
24	40	40	2.5	.430	34.7	0.16
12	40	43	2.5	.410	77.4	0.46
24	40	43	2.5	.430	59.9	0.27
12	42	42	2.5	....	....	0.44
24	42	42	2.5	....	....	0.22

Mother cultures of *L. helveticus*, incubated at 37° for 12 and 24 hours, were equally as active in 2.5-hour bulk cultures at 37° as in similar subcultures at 42°. Cultures of *Str. thermophilus* handled in a similar manner were shown to be equivalent in activity when mother cultures were grown at 37° for 12 or 24 hours. Cultures of both species of bacteria grown above 37° for 12 or 24 hours exhibited greatest variations in activity when the amounts of decrease in pH were compared. The differences in activity of cultures observed during the early hours of incubation were absent at the time 12-hour bulk cultures were compared. The capacity of 24-hour cultures to overtake the 12-hour mother cultures was more apparent with *Str. thermophilus* than *L. helveticus*. It may be related to the increased fer-

menting capacity of old cells observed by Rahn *et al.* (6) with old cells of *Str. lactis* beginning to multiply in a fresh medium.

Influence of low storage temperatures after the incubation of mother cultures upon the activity of 12-hour bulk cultures. The high degree of activity exhibited by mother cultures transferred at consecutive 24-hour intervals suggested that relatively short periods of incubation near the optimum temperature of growth for these bacteria could be used, followed by storage at temperatures below the optimum for growth. Mother cultures were incubated at 37° for 9, 12, or 16 hours, placed promptly at 0°, 20°, and 30° C., respectively, and stored until each culture was 24 hours old. The activities of bulk cultures grown at 37° for 12 hours are compared in table 4. Mother cultures of *L. helveticus* were stored at 0° and 20° until 24 hours old, following an initial incubation period of 12 to 16 hours at 37°, without reduction in growth or fermentation. Storage at 30°, however, resulted in decreased titratable acidities of the bulk cultures. Although Kopeloff *et al.* (4) reported that storage of other lactobacilli at 20° was less harmful than at lower temperatures, the data shown here indicate no marked differences in viability of *L. helveticus* during storage at 0° or 20° C.

TABLE 4

Differences in activity of bulk cultures after 12 hours at 37°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 37°C. for 9, 12 or 16 hours and then stored at 0°, 20°, or 30°C. until 24 hours old

Mother cultures					Activity of bulk cultures		
Incubation time at 37°C.	Storage: Time	Storage: Temp.	pH	Direct count	Direct count	pH	Titratable acidity
hours	*hours*	*°C.*		*millions/ml.*	*millions/ml.*		*per cent*
				L. helveticus			
9	15	0	4.55	674	1,069	4.54	0.76
12	12	0	4.23	727	863	4.47	0.85
16	8	0	4.09	739	876	4.45	0.85
12	12	20	4.21	887	790	4.47	0.87
16	8	20	4.14	1,015	879	4.42	0.87
12	12	30	4.02	623	....	4.50	0.75
16	8	30	3.93	830	....	4.47	0.75
				Str. thermophilus			
9	15	0	4.78	982	744	4.56	0.70
12	12	0	4.51	892	594	4.58	0.71
16	8	0	4.39	792	904	4.57	0.71
12	12	20	4.46	927	1,299	4.67	0.67
16	8	20	4.41	1,156	576	4.65	0.68
12	12	30	4.36	1,336	1,014	4.55	0.71
16	8	30	4.33	744	746	4.55	0.71

Cultures of *Str. thermophilus,* when treated in a manner similar to that

described for *L. helveticus,* were equally as active in bulk culture after storage at 0° and 20° C. as they were after being held at 30°. The initial incubation time of mother cultures before storage, whether 9, 12, or 16 hours, had no marked influence on the direct counts or acidities of the bulk cultures. Results of experiments (5) analogous to the one just described, except that bulk cultures were incubated at 30°, confirmed these observations and revealed that *L. helveticus* and *Str. thermophilus* could be stored at 0°, 10° or 20° C. for similar periods without reduction in activity.

Since a short storage period was harmless to either *L. helveticus* or *Str. thermophilus,* the effect of variations in storage time on growth and fermentation of mother cultures was studied. Cultures held at 42° for 12, 16, or 24 hours were continued at 20° for different periods. According to results shown in table 5, the incubation and storage time of mother cultures of *L. helveticus* and *Str. thermophilus* could be varied considerably without altering significantly the numbers of cells and titratable acidities of 12-hour bulk cultures. Cultures incubated at 42° for 12 or 24 hours could be stored

TABLE 5

Differences in activity of bulk cultures after 12 hours at 42°C.; inoculated with mother cultures of Lactobacillus helveticus or Streptococcus thermophilus grown at 42°C. for 12, 16, or 24 hours and then stored at 20°C. until 24, 48 or 96 hours old

Mother culture				Activity of bulk culture		
Incubation time at 42°C.	Storage at 20°C.	pH	Direct count	Direct count	pH	Titratable acidity
hours	*hours*		*millions/ml.*	*millions/ml.*		*per cent*
			L. helveticus			
12	12	4.15	516	965	4.27	0.95
16	8	4.00	659	766	4.25	0.93
12	36	3.89	893	1,013	4.27	0.98
12	84	3.78	784	1,006	4.14	1.01
24	24	3.71	886	889	4.35	0.93
24	72	3.69	601	641	4.30	0.93
12	none	....	1,579	1,309	4.10	0.97
24	none	....	864	1,028	4.19	0.93
			Str. thermophilus			
12	12	4.47	541	856	4.44	0.75
16	8	4.39	592	505	4.55	0.71
12	36	4.29	493	1,135	4.64	0.61
12	84	4.24	807	1,049	4.44	0.74
24	24	4.20	462	1,029	4.67	0.64
24	72	4.06	849	881	4.40	0.72
12	none	....	1,348	1,211	4.35	0.76
24	none	....	1,444	923	4.38	0.74

at 20° an additional 84 or 72 hours, respectively, without reduction in their activity. Storage under these conditions produced mother cultures equiva-

lent in bulk culture activity to cultures transferred every 12 or 24 hours near the optimum growth temperature. During storage at 20° a substantial number of cells die off, and mother cultures increase slowly in acidity. At this temperature, however, the effect of the developed acidity on the enzyme system of the majority of cells is probably diminished. According to Sarkaria and Hammer (7), true lactic acid bacteria grown in milk at different temperatures produced no measurable changes other than variations in total micro-population and acidity.

DISCUSSION

The results show that active cultures of *L. helveticus* and *Str. thermophilus* could be prepared with considerable latitude in incubation time and temperature. Variations within limits in methods of incubation of stock cultures can be employed with assurance that satisfactory growth and fermentation of cultures will be maintained. When large masses of cells, or bulk cultures, are required, similar variations in incubation time and temperature could be employed; and, in addition, if only a portion of the bulk culture is used, the remainder can be stored for short periods at low temperatures without loss in vitality. Thus time may be saved by the preparation of large quantities of bulk culture and punctuality in transferring may not be necessary, even though starter cultures may be needed every day or twice a day.

The results obtained in this study may not indicate the effects of these variations on the heat resistance of starter bacteria. This phase of the problem will be discussed in the following paper of this series.

SUMMARY

1. For mother cultures transferred successively at either 37°, 40°, or 42° C., the incubation time of *L. helveticus* and *Str. thermophilus* may be varied as much as 12 hours beyond an initial incubation period of 12 hours without harmful effect on culture activity. Cultures transferred every 12 or 24 hours produced similar populations and acidities when 12-hour bulk cultures made from them were compared at 37°.

2. Continuous incubation at 45° with transfers every 12 or 24 hours produced cultures of *Str. thermophilus* which were equivalent in activity to those grown at either 37°, 40°, or 42° C. Cultures of *L. helveticus* grew irregularly and poorly after successive transfers at 45°.

3. After incubation at 37° from 12 to 16 hours, or at 42° from 12 to 24 hours, cultures of *L. helveticus* could be stored at 0° to 20° C. until they were 96 hours old without reduction in their activity.

4. Cultures of *Str. thermophilus* incubated at 37° from 9 to 16 hours, or at 42° from 12 to 24 hours, could be continued at 0° or 20° C. until they

were 96 hours old without becoming less active than cultures transferred every 12 hours at 37° or 42° C.

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FACTORS AFFECTING THE ACTIVITY AND HEAT RESISTANCE OF SWISS CHEESE STARTER CULTURES. IV. EFFECT OF VARIATIONS IN TIME AND TEMPERATURE OF INCUBATION AND OF STORAGE ON HEAT RESISTANCE OF CULTURES*

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Improved methods of handling and preparing starter cultures of thermoduric lactic acid bacteria are useful to dairy manufacturers, especially to makers of Swiss cheese to whom the activity and heat resistance of the bacteria are of great importance. There is frequently a desire to modify the method of preparation of starter cultures, but the uncertainty of the effect the change may have on the vitality of the bacteria has limited attempts to vary customary procedures.

The present study was undertaken to determine to what extent time and temperature of incubation and of storage could be varied without loss in satisfactory activity and heat resistance of *Lactobacillus helveticus* and *Streptococcus thermophilus*, bacteria commonly used in dairy manufacture.

Previous investigations by us (13) have shown that incubation periods at a constant temperature near the optimum for growth, or in combination with storage at low temperatures, may be varied considerably without reduction of vitality of *L. helveticus* and *Str. thermophilus*. In these experiments the heat resistance of mother cultures was not determined. Brief studies by Elliker (4) demonstrated that cultures grown at 37° or 40° C. for 12 hours and promptly stored for the same time at 10° were equally as heat resistant as cultures transferred every 12 hours at 37° or 40° C.

Elliker and Frazier (5) observed that 12 and 16-hour cultures of *L. helveticus* developed better after a severe heat treatment than 7 or 8-hour cultures. They also established that greater resistance was exhibited by cultures incubated at 37° or 40° C. than by those grown at 30°, 35°, or 42° C. Six, 12 and 16-hour cultures of *Str. thermophilus* were equal in heat resistance when grown at 37°, but differences appeared when incubation was at 40°. Cultures grown at 30°, 35°, or 37° C. were more heat resistant than those incubated at 40° or 42° C.

Comprehensive investigations of factors affecting heat resistance of nonsporeforming bacteria appear in the literature. Such factors as temperature of incubation, age of culture, character of culture medium, and condi-

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ions of the heat treatment have been reviewed and studied by Anderson and Meanwell (1), Brown and Peiser (2), Claydon (3), Elliker (4), Elliker and Frazier (5), Fay (7), Peppler (12), and Robertson (14).

EXPERIMENTAL

Lactobacillus helveticus, Strain 39aW, and *Streptococcus thermophilus*, Strain C-3, the organisms used in studies by Elliker and Frazier (5, 6) and by us (13), were employed in the major portion of this investigation. In part of the work the heat resistance of the same strain of *L. helveticus* and Strain Mc of *Str. thermophilus*, both grown in symbiosis with a film yeast, *Candida krusei*, commonly called a "mycoderm," were compared with unassociated cultures of these bacteria.

Except for minor modifications the methods and apparatus used by Elliker and Frazier (5) were adopted. Mother cultures were grown in freshly autoclaved reconstituted skimmilk, designated as the "normal" medium. Since the heat resistance of *L. helveticus* is influenced by the quality of the medium, as shown by Elliker and Frazier (6), the organism also was grown in a "fortified" medium composed of freshly autoclaved reconstituted skimmilk and 0.1 per cent Neopeptone. *Str. thermophilus* was carried in the fortified medium in some studies. After seven to nine consecutive culture generations, final transfers were made to a larger quantity of medium to facilitate removal of samples for heat treatment and determinations of acidity. These cultures were designated as inoculating cultures. Heat resistance of cultures was measured in terms of rapid growth and acid production of subcultures in the normal skimmilk medium following a heat treatment simulating that encountered during Swiss cheese manufacture. Subcultures of *L. helveticus* were heated at 60° or 62° C. for 30 minutes; subcultures of *Str. thermophilus* were subjected to 62° or 64° C. for the same period. In most studies the higher temperature mentioned for each organism was adopted to emphasize differences in the activity of cultures. Plate counts were substituted for the direct microscopic method used by Elliker and Frazier to determine the number of viable cells directly before and after heating, and the rate of growth following the heat treatment. Plates were poured in triplicate using the medium of Kulp and White (9). Heat-treated subcultures were incubated at 37° to avoid the long chains which these bacteria form at higher temperatures. The amount of decrease in pH of the incubated culture during a definite period after heating was used as a measure of activity of surviving cells.

Influence of 12 and 24-hour successive transfers of mother cultures on heat resistance. The temperatures selected for incubation of the mother cultures of *L. helveticus* and *Str. thermophilus* were 37° and 40° C., since it had been found that heat resistant cultures were produced at both tem-

peratures, although the higher temperature was better under some conditions. Mother cultures of each organism were transferred every 12 and 24 hours. *L. helveticus* was grown in two different media. reconstituted skimmilk, or "normal" medium, and the same medium with "Neopeptone" added. Cultures of *Str. thermophilus* were grown in the fortified medium when it was observed that cultures carried in the normal medium developed a low resistance to heat.

TABLE 1

Influence of kind of culture medium and of successive transfers[1] of mother cultures of Lactobacillus helveticus and Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures			Heat-treated subcultures			
Incubation time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	°C.	
L. helveticus						
12 hrs. 37°	normal[2]	0.85	2.34	2.17	60	0.39
12 hrs. 37°	fortified[3]	0.89	2.47	2.23	60	0.48
24 hrs. 37°	normal	1.27	2.23	1.74	60	0.36
24 hrs. 37°	fortified	1.27	2.50	1.99	60	0.47
12 hrs. 40°	normal	1.13	2.66	2.46	62	0.53
12 hrs. 40°	fortified	1.09	2.08	2.02	62	0.53
24 hrs. 40°	normal	1.47	2.44	2.35	62	0.49
24 hrs. 40°	fortified	1.25	1.75	1.61	62	0.44
Str. thermophilus						
12 hrs. 37°	normal	0.71	0.614	0.407	62	1.22
24 hrs. 37°	normal	0.88	0.560	0.357	62	1.16
12 hrs. 40°	normal	0.72	0.585	0.220	62	0.98
12 hrs. 40°	fortified	0.90	0.720	0.490	62	1.26
24 hrs. 40°	normal	0.87	0.290	0.069	62	0.95
24 hrs. 40°	fortified	0.91	0.405	0.232	62	0.85

[1] Seven to nine consecutive transfers.
[2] Reconstituted skimmilk.
[3] Neopeptone-skimmilk medium.

The effect of successive transfers on the activity of heat-treated subcultures, as shown in table 1, varies not only with incubation temperature but also the quality of the medium. In order to limit the extensive data concomitant with hourly determinations of plate counts and acidity after heating, the plate counts directly before and after the heat treatment are reported, together with the amount of decrease in pH after six or seven hours. Mother cultures of *L. helveticus* grown in a fresh skimmilk medium at 40°, were more heat resistant than similar cultures developed at 37°. But in the fresh Neopeptone-skimmilk medium no significant differences in activity

after the heat treatment were exhibited by cultures grown either at 37° from 12 to 24 hours or at 40° for 12 hours. When *Str. thermophilus* was transferred every 12 and 24 hours at 37°, the cultures were equivalent to each other and more heat resistant than similar cultures carried at 40°. Growth in a fortified medium, however, provided 12-hour mother cultures at 40° which were equally as active after heating as those grown at 37°.

Influence of time and temperature of storage of mother cultures after incubation on heat resistance. Since the heat resistance of mother cultures was unaffected by certain limited variations in the incubation time during growth at a constant temperature, it was believed that such active cultures could be stored at low temperatures for limited periods without a reduction in heat resistance. *L. helveticus* and *Str. thermophilus* were incubated at 37° and 40° C. for periods of 6 to 24 hours and then promptly placed at 20°, 12°, or 4° C. for different periods of time. A constant temperature water bath was used for storage at 20°; an icebox having an average temperature of 12°, and a refrigerator with an average temperature of 4° provided the other means of storage.

The effect of storage time and temperature upon heat resistance of *L. helveticus* initially incubated at 37° for 12 or 24 hours is shown in table 2. Storage at 12° resulted in cultures with greater activity after a severe heat treatment than storage at 20° or 4° C. Storage time could be varied widely without significant reduction in activity of heat-treated subcultures. When *L. helveticus* was held at 37° for 12 hours, cultures could be stored at 12° for 60 hours and still be as heat resistant as the most active culture transferred every 12 hours at 37° C. Cultures incubated 24 hours at 37° and stored 48 hours at 12° were equally as resistant as those just described. When cultures were grown in a fresh Neopeptone-skimmilk medium before storage, they were more active after heating than similar cultures in the normal medium, but storage time was not prolonged beyond limits previously observed.

Storage of *Str. thermophilus*, after incubation at 37°, by methods similar to those just described for *L. helveticus*, resulted in heat resistant cultures at all low storage temperatures used, but, as the data in table 3 indicate, storage time at 12° could be varied more widely than at 4° or 20° C. Cultures which had been carried in a skimmilk medium with added Neopeptone showed more activity after the heat treatment than cultures which had been carried in the normal skimmilk medium. When *Str. thermophilus* was grown in the fortified medium, cultures carried at 37° for 12 or 24 hours followed by storage at 12° for 84 or 24 hours, respectively, as well as cultures grown 12 hours at 37° and continued for 36 hours at 4° C. were equivalent in activity after heating to cultures transferred serially at 37° in the normal medium.

According to data of table 4, *L. helveticus* incubated at 40° from 8 to 15 hours could be held for two days at low temperatures without decrease in heat resistance. When mother cultures were carried in the normal medium, incubation periods of 10 to 15 hours at 40° could be followed by 38 and 33 hours, respectively, at 20° or 12° C. In the medium with added Neopeptone, incubation periods at 40° for as little as 8 hours did not cause a reduction in heat resistance. The most active cultures in these experiments were equivalent in heat resistance to cultures transferred serially at either 37° or 40° C.

Previous investigations demonstrated that cultures of *Str. thermophilus* grown at 40° were less active after heat treatment than those carried at 37°. It could be expected that cultures stored at low temperatures after incubation at 40° would be no better than cultures transferred continuously at

TABLE 2

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incubation time at 37°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	°C.	
12	none	normal	0.85	2.34	2.17	60	0.39
	none	fortified	0.89	2.47	2.23	60	0.48
	36 hrs. 20°	normal	1.04	1.14	0.85	60	0.36
	36 hrs. 20°	fortified	1.07	1.45	1.21	60	0.39
	36 hrs. 12°	normal	0.97	2.48	2.55	62	0.50
	36 hrs. 12°	fortified	0.98	2.69	2.52	62	0.53
	12 hrs. 4°	normal	0.96	3.15	2.65	62	0.38
	36 hrs. 4°	normal	0.92	2.99	2.41	62	0.39
	84 hrs. 20°	normal	1.20	2.02	1.96	60	0.22
	84 hrs. 20°	fortified	1.30	2.17	2.04	60	0.28
	60 hrs. 12°	normal	1.22	2.02	2.16	62	0.46
	84 hrs. 12°	normal	1.06	1.89	1.69	62	0.37
	60 hrs. 4°	normal	0.97	2.62	2.45	62	0.43
	156 hrs. 4°	normal	0.91	2.93	2.57	62	0.31
24	none	normal	1.27	2.23	1.74	60	0.36
	none	fortified	1.27	2.50	1.99	60	0.47
	24 hrs. 20°	normal	....	2.90	2.56	60	0.36
	24 hrs. 20°	fortified	1.45	2.86	2.73	60	0.39
	24 hrs. 12°	normal	1.29	2.12	2.16	62	0.46
	24 hrs. 12°	fortified	1.21	2.04	1.79	62	0.41
	48 hrs. 12°	normal	1.46	2.26	2.21	62	0.46
	48 hrs. 4°	normal	1.41	3.20	2.74	62	0.43
	72 hrs. 20°	normal	1.35	1.81	1.28	60	0.22
	72 hrs. 20°	fortified	1.44	1.67	1.61	60	0.30
	72 hrs. 12°	normal	1.42	3.15	2.63	62	0.42
	144 hrs. 4°	normal	1.25	2.82	2.52	62	0.26

40°. This presumption was substantiated by the results presented in table 5. Only cultures carried in a medium better than fresh reconstituted skimmilk were as active after heating as the most heat resistant cultures developed at 37°. Cultures incubated in the fortified medium at 40° from 6 to

TABLE 3

Influence of kind of culture medium, incubation time at 37° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment

Inoculating cultures				Heat-treated subcultures			
Incubation time at 37°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Temp. of heating	Drop in pH six hours after heating
hours			per cent	millions/ml.	millions/ml.	°C.	
12	none	normal	0.72	0.614	0.407	62	1.05
	36 hrs. 20°	normal	0.82	0.640	0.482	62	1.04
	84 hrs. 20°	normal	1.05	0.750	0.378	62	1.12
	156 hrs. 20°	normal	1.01	0.414	0.309	62	0.85
	12 hrs. 12°	normal	0.74	0.727	0.060	64	0.85
	12 hrs. 12°	fortified	0.88	0.809	0.395	64	1.04
	36 hrs. 12°	normal	0.76	0.600	0.155	64	0.88
	36 hrs. 12°	fortified	0.91	0.635	0.133	64	1.13
	84 hrs. 12°	normal	0.84	0.550	0.025	64	0.99
	84 hrs. 12°	fortified	0.94	0.485	0.074	64	1.09
	156 hrs. 12°	normal	0.83	0.675	0.155	64	0.78
	156 hrs. 12°	fortified	0.96	0.780	0.174	64	0.99
	12 hrs. 4°	normal	0.73	0.770	0.043	64	0.53
	12 hrs. 4°	fortified	0.89	0.985	0.188	64	1.02
	36 hrs. 4°	normal	0.68	0.680	0.080	64	0.91
	36 hrs. 4°	fortified	0.82	0.770	0.229	64	1.13
	60 hrs. 4°	normal	0.74	0.612	0.103	64	0.50
10	14 hrs. 12°	normal	0.70	0.724	0.110	64	0.85
	14 hrs. 12°	fortified	0.82	0.867	0.380	64	1.11
24	none	normal	0.84	0.560	0.357	62	1.00
	24 hrs. 20°	normal	0.86	0.518	0.352	62	0.96
	72 hrs. 20°	normal	1.05	0.621	0.251	62	0.97
	24 hrs. 12°	normal	0.83	0.585	0.070	64	0.74
	24 hrs. 12°	fortified	0.95	0.590	0.250	64	1.05
	72 hrs. 12°	fortified	1.02	0.940	0.337	64	0.57
	48 hrs. 4°	normal	0.89	0.560	0.235	64	0.09

7 hours, and stored at either 12° or 4° C. from 42 to 41 hours, respectively, exhibited a remarkably high heat resistance. The increased resistance of *L. helveticus* and *St. thermophilus* after growth in fortified skimmilk media may, in a few instances, be due to greater numbers of cells surviving the heat treatment. In most instances marked differences in plate counts of survivors were not observed, regardless of the method of comparison used. Whether increases in heat resistance were the result of a greater accumula-

tion of mature, heat resistant cells in a favorable medium than in a poor medium, as Elliker and Frazier (5) suggest, or whether the growth stimulants merely improve the general healthiness of the cells was not revealed by this investigation.

TABLE 4

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Lactobacillus helveticus on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incubation time at 40°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
hours			per cent	millions/ml.	millions/ml.	
10	14 hrs. 20°	normal	1.05	2.42	2.15	0.49
10	38 hrs. 20°	normal	1.21	2.66	2.50	0.46
15	9 hrs. 20°	normal	1.27	2.02	2.50	0.46
15	33 hrs. 20°	normal	1.34	3.10	2.56	0.46
10	14 hrs. 12°	normal	0.87	2.76	2.18	0.48
10	14 hrs. 12°	fortified[1]	1.13	2.78	2.67	0.59
12	12 hrs. 12°	normal	1.03	2.37	2.30	0.51
12	12 hrs. 12°	fortified[1]	1.13	2.39	2.48	0.48
8	40 hrs. 12°	normal	0.86	2.36	1.83	0.36
8	40 hrs. 12°	fortified[1]	1.03	2.71	2.49	0.51
10	38 hrs. 12°	normal	1.00	2.42	2.21	0.52
15	33 hrs. 12°	normal	1.29	2.28	2.26	0.50
8	64 hrs. 12°	normal	0.95	2.45	2.12	0.42
15	57 hrs. 12°	normal	1.29	2.94	2.39	0.39
10	14 hrs. 4°	normal	0.92	2.26	2.28	0.37
15	9 hrs. 4°	normal	1.25	1.96	2.44	0.48
9	39 hrs. 4°	normal	0.87	2.63	2.09	0.44
9	39 hrs. 4°	fortified[1]	1.00	2.57	2.23	0.53
10	38 hrs. 4°	normal	0.92	2.39	2.25	0.37
15	33 hrs. 4°	normal	1.22	2.58	2.99	0.37
12	none	normal	1.13	2.66	2.46	0.39
12	none	fortified[2]	1.09	2.08	2.02	0.48
24	none	normal	1.47	2.44	2.35	0.36
24	none	fortified[2]	1.25	1.75	1.61	0.47

[1] Malt extract—skimmilk medium.
[2] Neopeptone—skimmilk medium.

Influence of a film yeast ("mycoderm") on heat resistance of bacteria grown in symbiosis with it. Several cultures in which the thermoduric lactic acid bacteria are associated with a film yeast, *Candida krusei*, sometimes called a "mycoderm," are used commonly by makers of Swiss cheese. In such cultures the "mycoderm" is known to increase viability of bacteria by reduction of the acidity (8, 11), and with some species of bacteria the production of lactic acid is stimulated (8, 10). Reports concerning the effect of a film yeast on thermal resistance of these bacteria have not been found. Comparisons of heat resistance were made between pure cultures of bacteria and the same cultures associated with a film yeast. *L. helveticus*, Strains 39aW and 39aW-my, and *Str. thermophilus*, Strains Mc and Mc-my,

TABLE 5

Influence of kind of culture medium, incubation time at 40° C., and storage time and temperature of mother cultures of Streptococcus thermophilus on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures				Heat-treated subcultures		
Incubation time at 40°	Storage time and temperature	Kind of medium	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH six hours after heating
hours			per cent	millions/ml.	millions/ml.	
8	16 hrs. 20°	normal	0.75	0.655	0.025	0.27
12	12 hrs. 20°	normal	0.79	0.520	0.025	0.27
15	9 hrs. 20°	normal	0.83	0.535	0.015	0.29
8	40 hrs. 20°	normal	0.81	0.570	0.065	0.52
15	33 hrs. 20°	normal	0.88	0.588	0.150	0.57
8	40 hrs. 12°	normal	0.72	0.589	0.153	0.66
15	33 hrs. 12°	normal	0.80	0.545	0.082	0.46
6	42 hrs. 12°	normal	0.66	0.653	0.064	0.53
8	64 hrs. 12°	normal	0.77	0.595	0.111	0.37
15	57 hrs. 12°	normal	0.84	0.555	0.100	0.37
8	40 hrs. 4°	normal	0.68	0.587	0.253	0.84
15	33 hrs. 4°	normal	0.79	0.661	0.031	0.28
7	41 hrs. 4°	normal	0.58	0.530	0.030	0.48
8	16 hrs. 4°	normal	0.71	0.520	0.060	0.60
15	9 hrs. 4°	normal	0.82	0.428	0.044	0.41
8	64 hrs. 4°	normal	0.65	0.730	0.044	0.52
12	60 hrs. 4°	normal	0.73	0.580	0.042	0.56
6	42 hrs. 12°	fortified[1]	0.82	0.645	0.333	1.25
7	41 hrs. 4°	fortified[1]	0.78	0.780	0.268	1.14
12	none	fortified[1]	0.90	0.720	0.490	1.26

[1] Neopeptone—skimmilk medium.

were selected. Strains 39aW-my and Mc-my were associated with *Candida krusei*. Methods of handling mother cultures described above were employed. Before the removal of samples for transfer to fresh media or for heat treatment, cultures were shaken to obtain a uniform mixture of film yeast and bacteria. The "mycoderm" was destroyed during the heat treatment; consequently, it did not interfere with plate counts or subsequent fermentation in the subcultures.

Results with *L. helveticus*, table 6, demonstrate that incubation for short consecutive periods at 37° C., and similar incubation periods followed by storage at 20° C. for 36 hours produced no differences in heat resistance between symbiotic and pure cultures. But when starters were incubated for 72 to 96 hours at 25° or 12-hour (37°) cultures were stored for 154 hours at 20°, the associated cultures were significantly more heat resistant than the pure cultures. It was observed that bacteria nearest the surface growth of the film yeast were far more active after heat treatment than bacteria farthest removed from the top of the mixed culture.

Similar treatment of *Str. thermophilus* also revealed that associated cul-

TABLE 6

Influence of incubation time and temperature of mother cultures of Lactobacillus helveticus (39aW) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 62° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
39aW	12 hrs. 37°	0.89	2.67	2.15	0.30
39aW-my	12 hrs. 37°	0.92	1.64	1.17	0.30
39aW	12 hrs. 37° 36 hrs. 20°	0.98	1.93	1.97	0.43
39aW-my	12 hrs. 37° 36 hrs. 20°	1.17	2.07	1.98	0.43
39aW	72 hrs. 25°	1.20	1.58	1.71	0.37
39aW-my	72 hrs. 25°	1.24	1.20	1.76	0.44
39aW	96 hrs. 25°	1.42	1.90	1.80	0.45
39aW-my	96 hrs. 25°	1.37	2.11	1.64	0.53
39aW	14 hrs. 37° 154 hrs. 20°	1.27	2.80	2.80	0.41
39aW-my	14 hrs. 37° 154 hrs. 20°				
*(a)		1.18	2.18	2.36	0.68
(b)		1.56	2.01	2.06	0.55
(c)		1.28	2.12	1.89	0.62

* (a) Subculture from topmost quarter of inoculating culture.
(b) Subculture from bottom quarter of inoculating culture.
(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

tures possessed greater activity after heating than pure cultures of this streptococcus. As shown in table 7 heat resistant mixed cultures were obtained when they were carried at 37° for 12 hours or at 25° for 72 hours. The influence of the "mycoderm" was not overcome by the number of successive transfers at 37° used here. In some instances increased activity may be attributable to higher numbers of survivors. It is also evident from data of table 7 that bacteria in the vicinity of "mycoderm" growth were more active after heat treatment than those farthest from the surface film.

The role played by *Candida krusei* resulting in enhanced heat resistance of these lactic starter bacteria will be discussed in another paper.

A summary of variations in time and temperature of incubation and storage which provided starter cultures of equivalent and maximum heat resistance appears in table 8.

DISCUSSION

The data support the belief that a variety of modifications can be applied to methods for handling starter cultures of thermoduric bacteria, depending

TABLE 7

Influence of incubation time and temperature of mother cultures of Streptococcus thermophilus (Mc) grown with a "mycoderm" (my) on the activity of subcultures at 37° C. following heat treatment at 64° C. for 30 minutes

Inoculating cultures			Heat-treated subcultures		
Type of mother culture	Incubation time and temperature	Titratable acidity	Plate count before heating	Plate count after heating	Drop in pH seven hours after heating
		per cent	millions/ml.	millions/ml.	
Mc	12 hrs. 37°	0.62	0.560	0.075	0.42
Mc-my	12 hrs. 37°	0.64	0.565	0.175	0.60
Mc	72 hrs. 25°	0.71	0.435	0.205	1.17
Mc-my	72 hrs. 25°	0.69	0.545	0.390	1.25
Mc	96 hrs. 25°	0.68	0.484	0.096	1.04
Mc-my	96 hrs. 25°	0.64	0.449	0.098	1.04
Mc	12 hrs. 37° 36 hrs. 20°	0.61	0.448	0.010	0.45
Mc-my	12 hrs. 37° 36 hrs. 20°	0.64	0.599	0.020	0.48
Mc	14 hrs. 37° 154 hrs. 20°	0.83	0.628	0.318	0.30
Mc-my	14 hrs. 37° 154 hrs. 20°				
*(a)		0.75	0.425	0.090	0.89
(b)		0.91	0.505	0.085	0.11
(c)		0.78	0.415	0.105	0.41

* (a) Subculture from topmost quarter of inoculating culture.
(b) Subculture from bottom quarter of inoculating culture.
(c) Subculture from inoculating culture previously thoroughly shaken to mix growth of top and bottom areas.

upon incubation temperature used, storage temperature available, and quality of the culture medium employed, without lessening the activity and heat resistance of the bacteria.

L. helveticus and *Str. thermophilus* may be grown in a good medium such as reconstituted skimmilk at a common temperature, 37°, to develop cultures of high heat resistance. If a better culture medium, one with added accessory substances is used, the heat resistance of cultures is not only assured but actually increased. This modification of methods for handling starter may be adopted when higher than usual cooking temperatures of curd are employed to avoid undesirable gas formation in Swiss cheese. The results of this investigation indicate that under such circumstances starter of great heat resistance can be produced by improving the culture medium, by incubation at 40° instead of 37° C., or by combining both procedures. In the case of *Str. thermophilus,* however, growth at 40° requires a medium of highest quality, and more careful control of incubation time is necessary to provide cultures of proper maturity. The results again emphasize the importance of a suitable and uniform starter culture medium, suggesting adoption of a definite plan of selection of high quality milk for this purpose.

TABLE 8

A summary of variations in time and temperature of incubation and storage which provided cultures of equivalent and maximum heat resistance

Starter	Incubation time and temperature	Quality of skimmilk medium
L. helveticus strain 39aW	12 hrs. 37° + 60 hrs. 12° 24 hrs. 37° + 48 hrs. 12° 10–15 hrs. 40° + 38 hrs. 20° or 12°	normal*
	12–24 hrs. 37° or 40° 12 hrs. 37° + 36 hrs. 12° 8– 9 hrs. 40° + 40 hrs. 12° or 4° 10–12 hrs. 40° + 12 hrs. 12° 15 hrs. 40° + 9 hrs. 4°	fortified**
Str. thermophilus strain C-3	12–24 hrs. 37° 12 hrs. 37° + 84 hrs. 20° 12 hrs. 37° + 36 hrs. 12°	normal*
	12 hrs. 37° or 40° 10–12 hrs. 37° + 84 hrs. 12° 12 hrs. 37° + 36 hrs. 4° 24 hrs. 37° + 24 hrs. 12° 6– 7 hrs. 40° + 42 hrs. 12° or 4°	fortified**

* Mother cultures grown in reconstituted skimmilk medium.
** Cultures carried in skimmilk medium with accessory substances added.

Results obtained with cultures transferred every 24 hours at either 37° or 40° C. suggest that starter cultures of *L. helveticus* and *Str. thermophilus* need not be transferred twice daily, as it is done in many cheese factories, to obtain heat resistant bacteria. The preparation of bulk starter for Swiss cheese need not be a daily task. With adequate incubation and storage facilities, bulk starter grown at 37° for 12 or 24 hours, for example, can be held from one to two days in a factory cold room. Thus enough starter can be prepared to supply the need for two days' manufacture. When cheese is made twice daily, starter for both lots can be prepared at the same time, a portion to be used in the morning and the remainder held in the cold room for use in cheese made in the afternoon.

The increased heat resistance of *L. helveticus* and *Str. thermophilus* grown in association with the film yeast, *Candida krusei,* revealed an aspect of symbiosis not recognized previously. Limited studies suggest the possibility that heat resistant cultures of these bacteria may be developed at temperatures considerably below the optimum for thermoduric bacteria, such as 30° C. It was demonstrated that bacteria near the "mycoderm" pellicle were more active after heating than bacteria from the same culture but at the lowest level, and that the beneficial influence of the film yeast extended for some distance below the surface of the culture. These results suggest that heat resistant cultures of these bacteria may be obtained by methods other than those customarily employed; such as, growing asso-

ciated cultures in thin layers of media, or in media with increased surface area.

Although the data have been discussed only in regard to their use by workers in the Swiss cheese industry, others who prepare cultures of thermoduric lactic acid bacteria required to grow rapidly after severe heat treatments may find helpful modifications of methods for handling such bacteria.

SUMMARY

1. Successive transfers every 12 and 24 hours at 37° and 40° C. resulted in more heat resistant cultures of *L. helveticus* at 40° than at 37° when mother cultures were grown in freshly reconstituted skimmilk. In a medium with added accessory substances, such as Neopeptone or malt extract, 12 to 24 hours at 37° and 12 hours at 40° gave best results.

2. Consecutive transfers of *Str. thermophilus* at a constant temperature led to most active cultures when incubation in the fortified medium varied from 12 to 24 hours at 37°; but at 40° highest resistance to heat was shown by 12-hour cultures carried in the fortified medium.

3. Storage at 12° was least harmful to *L. helveticus* following incubation at 37°. Greatest heat resistance was observed when 12-hour cultures at 37° were held at 12° no longer than 60 hours, and 24-hour cultures at 37° could be stored for 48 hours at 12°. Improvement of the skimmilk medium with Neopeptone increased slightly the heat resistance of stored mother cultures.

4. *Str. thermophilus* grown in the fortified medium at 37° for 10 to 12 hours and then stored 84 hours at 12° or 36 hours at 4°, and 24-hour cultures at 37° held 24 hours at 12° were equivalent in heat resistance to cultures transferred every 12 hours at 37°. Storage of cultures at 12° was better than at either 4° or 20° C.

5. After incubation at 40° for 8 to 15 hours, *L. helveticus* could be stored at 20°, 12°, or 4° C. until cultures were 48 hours old. Storage at 12° appeared to be least harmful to mother cultures.

6. When grown at 40° C. *Str. thermophilus* gave heat resistant cultures only when Neopeptone was added to the skimmilk medium. Incubation at 40° for 6 to 7 hours followed by storage at either 12° or 4° C. until cultures were 48 hours old resulted in cultures as active after heating as those transferred serially at 37°.

7. Mixed cultures of the film yeast, *Candida krusei,* and *L. helveticus* exhibited greater heat resistance than pure cultures of this bacterium after incubation at 25° C. for 72 to 96 hours, and also when incubation at 37° C. for 12 hours was followed by storage at 20° C. for 36 hours. Frequent transfer of associated cultures at 37° reduced the influence of the "mycoderm"; these cultures were equal in heat resistance to pure cultures of the bacteria carried under similar conditions.

8. *Str. thermophilus,* Mc, grown with "mycoderm" was more active after heating than pure cultures of the bacteria carried under similar conditions. Numerous successive 12-hour transfers of the associated culture at 37° did not decrease the influence of the film yeast.

9. In old cultures bacteria nearest the "mycoderm" film grew and fermented better following heat treatment than bacteria in areas farthest removed from the pellicle formed by the film yeast.

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THE LIMIT OF ERROR OF THE SIMPLIFIED VACUUM SOLIDS TEST AS APPLIED TO ICE CREAM MIX, EVAPORATED AND SWEETENED CONDENSED MILK

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INTRODUCTION

A Simplified Vacuum Solids Test for determining total solids in ice cream mix, condensed and evaporated milk has been developed by the Department of Dairy Manufactures of Texas Technological College. This method was devised in order to provide the dairy industry with a method for the estimation of total solids, which is accurate enough to meet the requirements of the commercial operator, rapid enough to be of commercial value, cheap enough to be available to all who wish to perform analysis for total solids, and simple enough to be performed by the average laboratory employee.

The purpose of this work was to determine the most satisfactory procedure for operating the Simplified Vacuum Solids Test; also to determine its accuracy as compared to the Official Method for determining total solids in evaporated milk, sweetened condensed milk, and to the adapted Official Method for ice cream mix.

The Simplified Vacuum Solids Test was originally designed in 1933 by Professor K. M. Renner, Head of the Department of Dairy Manufactures, Texas Technological College. Dr. William L. Ray, formerly of the Chemistry Department of Texas Technological College, offered valuable suggestions and advice on the construction of the original apparatus.

The first application of the test was made in the analysis for total solids in egg yolk. Numerous changes from the original design and procedure have been made in order to make the test more adaptable to liquid dairy products.

A number of commercial plants have adopted the Simplified Vacuum Solids Test. Until the present work was performed, there were no data available showing a statistical comparison of the Simplified Vacuum Solids Test to the Official Method.

REVIEW OF LITERATURE

The accuracy of several methods commonly used for testing condensed milk, evaporated milk and ice cream mix for total solids has been studied by previous investigators. Among the methods commonly used are the Mojonnier, Refractometric, and various adaptations of the Official.

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In studying the accuracy of methods for testing sweetened condensed milk, Fisher and Rice (2) compared results obtained by the Official method to the Mojonnier and a Modified Official method which they devised. These workers tested twenty samples of sweetened condensed milk by all three methods, and found that in all but one instance the Official method yielded the lowest results. The Mojonnier method gave the highest results on two-thirds of the tests. On these twenty samples the Mojonnier results averaged 0.57 per cent higher than the Official, whereas the Modified method averaged only 0.3022 per cent higher.

Fisher and Watts (3) made a study of some of the methods used in testing ice cream for total solids. They compared the adapted Official method to the Modified method recommended by Fisher and Rice (2) and to the Mojonnier method. They tested twelve samples of ice cream by each of the methods listed above. On all but two samples the adapted Official method yielded the lowest results, and the Mojonnier method the highest. The Mojonnier results averaged 0.44 per cent higher than the adapted Official, and the Modified method results averaged 0.23 per cent higher. These observations substantiated the results obtained by Fisher and Rice (2) in their work on sweetened condensed milk.

Rice and Miscall (6) in studying the Refractometric method of determining total solids in sweetened condensed milk, found that if accurate results are to be obtained the determinations must be made within a few hours after the batch is drawn because of the crystallizing tendency of lactose. These workers devised a formula by which total solids could be calculated from the refractive index, but they pointed out limitations of the reliability of the method in that the casein and albumen cannot be completely precipitated.

Menefee and Overman (4) also studied the Refractometric method of testing condensed and evaporated milk for total solids, using a copper sulphate solution to precipitate the protein. These investigators developed a formula for computing the total solids from the Refractometer reading of these products. They tested sixteen samples of condensed milk and found a standard error of estimate of 0.4641, with the greatest error on any sample 0.78. Twenty-five samples of evaporated milk were tested, and the results showed a standard error of estimate of 0.49, with the largest single error being 0.97.

LABORATORY EQUIPMENT

The following laboratory equipment is required for assembling the Simplified Vacuum Solids Testing Device:

(1) 1—1000-ml. Pyrex Beaker
(2) 1—1000-ml. Suction Flask
(3) Pyrex Test Tubes 7″ long and 1″ in diameter

(4) 1—Glass Tee
(5) 1—Glass L
(6) 5—Ft. of 7-mm. Glass Tubing
(7) 1—2-oz. Jar and Mercury for Mercury Well
(8) 1—Aspirator
(9) 1—No. 8 Two-hole Rubber Stopper
(10) 1—No. 10 Two-hole Rubber Stopper
(11) 1—Bunsen Burner
(12) 3—Ft. of Heavy Rubber Tubing of suitable size to fit Glass Tee, Suction Flask and Aspirator

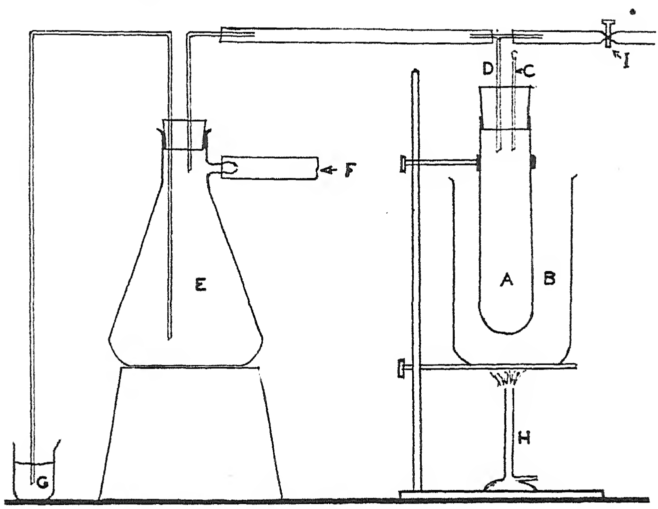

FIG. 1. Simplified vacuum solids testing apparatus.
A—1″×7″ pyrex test tube for sample.
B—1000-ml. beaker, water bath.
C—Thistle tube.
D—Glass T.
E—500-ml. suction flask.
F—Suction tubing to aspirator (suction pump).
G—Mercury well.
H—Bunsen burner—Heat source.
I—Pinch cock.

The materials used and the method of assembling the apparatus are shown in the accompanying illustration. The assembled system must be free from air leaks, and the water pressure sufficient to maintain a 24-inch vacuum as measured on the mercury column.

MATERIAL TESTED

All of the ice cream mix and sweetened condensed milk samples tested in this work were obtained from the College Creamery. Commercial samples of evaporated milk were purchased from local retail stores. Prior to weigh-

ing and testing, the samples were kept at a temperature of about 40° F. to minimize bacterial action and acid development. No sample was held longer than two days, that being the length of time required to perform the series of fifty tests which were performed on each sample.

PREPARATION OF SAMPLES

None of the sweetened condensed milk tested in this work had been homogenized, whereas all of the ice cream mix and evaporated milk samples had been homogenized. The sweetened condensed skim milk samples were prepared according to the recommendations of the Official method (1) for total solids determination. An attempt was made to prepare the sweetened condensed whole milk samples in the same manner, but was unsuccessful because the butterfat churned when the warm diluted sample was agitated. The sweetened condensed whole milk samples were maintained at a temperature of about 50° F. while being diluted with an equal weight of cold distilled water, stirred and mixed. The homogenized ice cream mix and evaporated milk samples required no preliminary treatment other than mixing. All samples were thoroughly mixed by stirring and pouring several times before weighing each sample.

TESTING PROCEDURE

A large (1″ × 7″) test tube is placed on the scale pan of an analytical balance, and is held in an upright position by a wire clasp designed for the purpose. One to two grams of the sample is weighed into the test tube. The test tube containing the sample is then placed in the 1000-ml. beaker which contains boiling distilled water. The test tube and beaker are supported by a ring stand as shown in the illustration. The two-hole rubber stopper, into which is inserted the thistle tube and suction line, is seated in the test tube, and the water then turned into the aspirator.

As the vacuum is created in the test tube, the sample boils vigorously and spreads in a thin film over the interior of the test tube. Care must be exercised at this point to prevent any of the sample from escaping into the suction line. This is controlled by using proper volume and dilution of sample, and if necessary temporarily breaking the vacuum. The sample is thus heated for fifteen minutes, then removed from the boiling water, dried thoroughly, cooled in a desiccator and weighed. The per cent total solids is calculated by dividing the weight of residue by the weight of sample and multiplying by 100. A proper correction must be made according to the amount of the original dilution.

EXPERIMENTAL

A large number of preliminary analyses was performed in an attempt to improve on the procedure previously recommended by Renner (5) in

which the weighings were performed on a Torsion balance. In the operation of the Simplified Vacuum Solids Test, the best results were obtained when a sample weighing from one to two grams was used, diluted in such a manner as to contain between 18 per cent and 35 per cent total solids, heated at 212° F. under at least a 24-inch vacuum for fifteen minutes, and cooled in a desiccator before weighing.

In order to determine the accuracy of the Simplified Vacuum Solids Test, groups of fifty tests for total solids were performed on each of the following products: Evaporated Milk, Ice Cream Mix, Sweetened Condensed Whole Milk and Sweetened Condensed Skim Milk.

Total solids determinations were also made by the Official method (1) or in the case of ice cream mix, an adapted Official method (2). The error of the Simplified Vacuum Solids method was measured from the average of four Official results on each sample.

For the purpose of measuring the accuracy of the results, the following statistical methods were applied to all groups of tests:

1. The arithmetic mean of error was determined on the error and on the total solids.
2. The standard deviation was determined on the error and on the total solids.
3. The coefficient of variation was determined on the total solids.

The accuracy of the Simplified Vacuum Solids Test as compared to the Official method for determining the total solids in evaporated milk, ice cream mix, sweetened condensed whole milk and sweetened condensed skim milk is shown in tables 1, 2, 3 and 4, respectively.

DISCUSSION AND CONCLUSIONS

One of the greatest difficulties in the operation of the Simplified Vacuum Solids Test is the prevention of the loss of small particles of sample into the suction line. This difficulty can be controlled by using a small sample (one or two grams) which has been diluted so as to contain between 18 per cent and 35 per cent total solids. Low solids samples have more tendency to be drawn into the suction line, whereas high solids samples having a high viscosity do not spread in a thin uniform film over the interior of the surface of the test tube, and thus have less evaporation surface.

The arithmetic mean of the error and of the total solids was determined in order to show the simple average of the error. The standard deviation was used in comparing the error of the results, because it is a measure of absolute variability. The coefficient of variability was also calculated, since it shows the ratio of the standard deviation to the arithmetic mean.

In estimating the total solids in evaporated milk, the Simplified Vacuum Solids method compared favorably with other methods in use, in that the arithmetic mean of error was 0.1529.

TABLE 1

Evaporated milk

Official T. S. = 26.57%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	2.0539	.5487	26.72	.15
2	2.0137	.5369	26.66	.09
3	2.0145	.5380	26.71	.14
4	1.9976	.5321	26.64	.07
5	2.0113	.5356	26.62	.05
6	2.0065	.5340	26.61	.04
7	1.9418	.5200	26.77	.20
8	1.9404	.5190	26.74	.17
9	1.8876	.5035	26.67	.10
10	1.8689	.4989	26.69	.12
11	1.8749	.5017	26.75	.18
12	1.8705	.5005	26.75	.18
13	1.8896	.5058	26.74	.17
14	1.9391	.5193	26.78	.21
15	2.0827	.5570	26.74	.17
16	2.1160	.5654	26.72	.15
17	2.0820	.5548	26.64	.07
18	2.0396	.5462	26.78	.21
19	2.0342	.5443	26.76	.19
20	2.0576	.5506	26.76	.19
21	1.9757	.5277	26.71	.14
22	2.0303	.5412	26.66	.09
23	1.9553	.5251	26.85	.28
24	2.0012	.5347	26.72	.15
25	1.9779	.5290	26.75	.18
26	1.9701	.5278	26.78	.21
27	1.9333	.5176	26.77	.20
28	1.9750	.5294	26.78	.21
29	1.9638	.5259	26.78	.21
30	2.1213	.5647	26.62	.05
31	2.0613	.5500	26.68	.11
32	2.0344	.5427	26.68	.11
33	2.0046	.5333	26.60	.03
34	2.0279	.5412	26.69	.12
35	1.9319	.5172	26.77	.20
36	1.9926	.5334	26.77	.20
37	1.9467	.5208	26.75	.18
38	1.9563	.5237	26.76	.19
39	1.9365	.5090	26.75	.18
40	1.8891	.5058	26.77	.20
41	1.9449	.5202	26.75	.18
42	1.9555	.5231	26.75	.18
43	1.9386	.5181	26.73	.16
44	1.9120	.5128	26.82	.25
45	1.9318	.5171	26.76	.19
46	1.8735	.5030	26.84	.27
47	2.1404	.5690	26.58	.01
48	2.0434	.5456	26.70	.13
49	2.0250	.5386	26.60	.03
50	2.1125	.5655	26.72	.15

Statistical analysis of results in table 1

1. The arithmetic mean of error = 0.1529.
2. The standard deviation of the error = 0.0624.
3. The arithmetic mean of the total solids = 26.72.
4. The standard deviation of the total solids = 0.0624.
5. The coefficient of variation of the total solids = 0.2335.
6. No minus errors were obtained.
7. The range of error was from 0.01 to 0.28.
8. Ninety-six per cent of the results were within 0.25 per cent of the Official.

TABLE 2

Ice cream mix

Official T. S. = 36.87%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1645	.4360	37.44	.57
2	1.0222	.3801	37.18	.31
3	1.0284	.4041	37.33	.46
4	1.0298	.3908	37.94	1.07
5	1.0496	.3900	37.16	.29
6	1.0554	.3936	37.29	.42
7	1.1056	.4110	37.18	.31
8	1.0408	.3863	37.12	.25
9	1.0026	.3732	37.22	.35
10	1.0212	.3818	37.39	.52
11	1.0438	.3894	37.31	.44
12	1.0196	.3823	37.49	.62
13	1.0076	.4764	37.36	.49
14	1.0107	.3787	37.47	.60
15	1.0130	.3755	37.07	.20
16	1.0032	.3779	37.67	.80
17	.9979	.3740	37.48	.61
18	.9899	.3657	36.94	.07
19	1.0109	.3759	37.14	.27
20	1.0651	.3965	37.29	.42
21	1.0214	.3819	37.39	.52
22	1.0567	.3950	37.38	.51
23	1.0170	.3799	37.35	.48
24	1.0442	.3889	37.24	.37
25	1.0653	.3993	37.48	.61
26	1.0620	.3936	37.07	.20
27	.9936	.3733	37.57	.70
28	1.0250	.3855	37.71	.84
29	1.0198	.3823	37.48	.61
30	.9857	.3681	37.45	.58
31	.9973	.3725	37.36	.49
32	1.0210	.3815	37.36	.49
33	1.0301	.3887	37.73	.86
34	1.0252	.3830	37.41	.54
35	1.0221	.3804	37.21	.34
36	1.0132	.3811	37.61	.74
37	1.0060	.3788	37.65	.78
38	.9908	.3695	37.29	.42
39	.9901	.3719	37.56	.69
40	.9717	.3649	37.55	.68
41	1.0038	.3742	37.28	.41
42	.9723	.3639	37.43	.56
43	.9643	.3590	37.23	.36
44	1.0105	.3790	37.49	.62
45	.9943	.3675	36.96	.09
46	.9673	.3607	37.29	.42
47	.9504	.3589	37.76	.89
48	.9994	.3755	37.57	.70
49	1.0026	.3762	37.52	.65
50	.9647	.3580	37.11	.24

Statistical analysis of results in table 2

1. The arithmetic mean of the error = 0.5090.
2. The standard deviation of the error = 0.1521.
3. The arithmetic mean of the total solids = 37.38.
4. The standard deviation of the total solids = 0.2261.
5. The coefficient of variation of the total solids = 0.6032.
6. No minus errors occurred in the results.
7. The range of error was from 0.07 to 1.07.
8. Twelve per cent of the results were within 0.25 per cent of the adapted Official.
9. Fifty per cent of the results were within 0.50 per cent of the adapted Official.
10. Ninety-eight per cent of the results were within 1 per cent of the adapted Official.
11. Only one sample had an error in excess of 1 per cent.

TABLE 3

Sweetened condensed whole milk

Official T. S. = 70.86%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.1106	.3961	71.34	.48
2	1.1004	.3918	71.22	.36
3	1.0915	.3905	71.56	.70
4	1.1130	.3966	71.26	.40
5	1.0959	.3906	71.28	.42
6	1.0982	.3909	71.18	.32
7	1.0640	.3795	71.34	.48
8	1.0743	.3815	71.02	.16
9	1.0614	.3772	71.08	.22
10	1.0601	.3780	71.32	.46
11	1.0776	.3834	71.16	.30
12	1.0439	.3721	71.30	.44
13	1.0458	.3732	71.36	.50
14	1.1027	.3942	71.48	.62
15	1.0841	.3875	71.48	.62
16	1.0618	.3781	71.20	.34
17	1.0854	.3880	71.50	.64
18	1.1314	.4017	71.00	.14
19	1.0372	.3700	71.34	.48
20	1.0340	.3705	71.66	.80
21	1.0289	.3664	71.22	.36
22	1.0313	.3662	71.02	.16
23	1.0531	.3784	71.86	1.00
24	1.0423	.3693	70.86	.00
25	1.0397	.3682	70.82	−.04
26	1.0332	.3704	71.70	.84
27	1.0489	.3726	71.04	.18
28	1.0286	.3667	71.30	.44
29	1.0418	.3720	71.42	.56
30	1.0392	.3691	71.04	.18
31	1.0503	.3760	71.58	.72
32	1.0377	.3672	70.78	−.08
33	1.0645	.3775	70.92	.06
34	1.0320	.3680	71.32	.46
35	1.0468	.3724	71.16	.30
36	1.0268	.3649	71.08	.22
37	1.0595	.3773	71.22	.36
38	1.0574	.3768	71.26	.40
39	1.0571	.3758	71.12	.26
40	1.0462	.3718	71.08	.22
41	1.0325	.3683	71.34	.48
42	1.0363	.3695	71.32	.46
43	1.0411	.3778	71.42	.56
44	1.0391	.3701	71.24	.38
45	1.0494	.3747	71.42	.56
46	1.0314	.3673	71.22	.36
47	1.0940	.3910	71.48	.62
48	1.0637	.3779	71.06	.20
49	1.0500	.3727	71.00	.14
50	1.0211	.3630	71.10	.24

Statistical analysis of results in table 3

1. The arithmetic mean of the error = 0.3944.
2. The standard deviation of the error = 0.2284.
3. The arithmetic mean of the total solids = 71.25.
4. The standard deviation of the total solids = 0.2278.
5. The coefficient of variation of the total solids = 0.3197.
6. Four per cent of the results had a minus error.
7. The range of error of the results was from minus 0.08 to plus 1.00.
8. Thirty per cent of the results were within 0.25 per cent of the Official.
9. Seventy-six per cent of the results were within 0.50 per cent of the Official.
10. All of the results were within 1 per cent of the Official.

TABLE 4

Sweetened condensed skim milk

Official T. S. = 70.96%

Sample No.	Wt. of sample	Wt. of residue	% T. S. determined	Error
1	1.0265	.3693	71.96	1.00
2	1.0176	.3697	72.66	1.70
3	1.0274	.3695	71.92	.96
4	1.0227	.3665	71.70	.74
5	1.0384	.3702	71.70	.74
6	1.0245	.3683	71.90	.94
7	1.0292	.3700	71.90	.94
8	1.0310	.3704	71.86	.90
9	1.0202	.3676	72.06	1.10
10	1.0263	.3711	72.32	1.36
11	1.0299	.3699	71.84	.88
12	1.0359	.3741	72.22	1.26
13	1.0320	.3750	72.66	1.70
14	1.0221	.3680	72.00	1.04
15	1.0118	.3641	71.98	1.02
16	1.0327	.3728	72.20	1.24
17	1.0477	.3799	73.28	2.32
18	1.0335	.3733	72.24	1.28
19	1.0473	.3805	72.66	1.70
20	1.0224	.3766	71.74	.78
21	1.0535	.3778	71.72	.76
22	.9979	.3583	71.82	.86
23	.9965	.3569	71.64	.68
24	1.0570	.3787	71.66	.70
25	1.0377	.3731	71.92	.96
26	1.0225	.3650	71.40	.44
27	1.0386	.3715	71.54	.58
28	1.0663	.3859	72.38	1.42
29	1.0353	.3720	71.86	.90
30	1.0385	.3705	71.36	.40
31	1.0062	.3649	72.64	1.68
32	1.0573	.3813	72.12	1.16
33	1.1344	.4026	70.98	.02
34	1.0102	.3681	72.88	1.92
35	1.0207	.3706	72.58	1.62
36	1.0266	.3681	71.72	.76
37	1.0291	.3717	72.24	1.28
38	1.0253	.3745	73.06	2.10
39	1.0352	.3744	72.34	1.38
40	1.0134	.3702	73.06	2.10
41	1.0307	.3725	72.28	1.32
42	1.0296	.3732	72.50	1.54
43	1.0214	.3679	72.04	1.08
44	1.0418	.3760	72.18	1.22
45	1.0418	.3787	72.70	1.74
46	1.0394	.3813	73.40	2.44
47	1.0333	.3893	75.36	4.40
48	1.0335	.3828	74.08	3.12
49	1.0269	.3706	72.18	1.22
50	1.0294	.3677	71.44	.48

Statistical analysis of results in table 4

1. The arithmetic mean of the error = 1.2776.
2. The standard deviation of the error = 0.7214.
3. The arithmetic mean of the total solids = 72.24.
4. The standard deviation of the total solids = 0.7220.
5. The coefficient of the variation of the total solids = 0.9996.
6. No minus errors occurred in the results.
7. The range of error of the results was from 0.02 to 4.40.
8. Only one result, or 2 per cent, was within 0.25 per cent of the Official.
9. Eight per cent of the results were within 0.50 per cent of the Official.
10. Forty-two per cent of the results were within 1 per cent of the Official.
11. Fifty-eight per cent of the results varied more than 1 per cent from the Official.

Menefee and Overman (4) applied the Refractometric method to evaporated milk, and reported a standard error of estimate of 0.49.

The Simplified Vacuum Solids Test applied to ice cream mix yielded an arithmetic mean of error of 0.5090. Fisher and Watts (3) compared the Mojonnier method to the Official, and reported an average variation on the Mojonnier of 0.4830.

Sweetened condensed whole milk samples tested by the Simplified Vacuum Solids method showed an arithmetic mean of error of 0.3944. Fisher and Watts (3) tested sweetened condensed whole milk by the Mojonnier method, and reported an arithmetic mean of error of 0.57, and Menefee and Overman (4) using the Refractometric method reported an arithmetic mean of error of 0.4641.

The Simplified Vacuum Solids method results were less satisfactory on sweetened condensed skim milk than on the other products. The arithmetic mean of error was 1.2776. The Mojonnier and Refractometric methods can be applied to sweetened condensed skim milk and sweetened condensed whole milk with about equal accuracy.

The results obtained by the Simplified Vacuum Solids method compared favorably with results obtained from other methods by previous workers when the comparisons were made by statistical devices commonly used for measuring accuracy.

The Simplified Vacuum Solids Test can be performed in twenty to twenty-five minutes, which compares favorably with the time required for other common total solids tests.

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EVIDENCE FOR THE PRESENCE OF SMOOTH MUSCLE ELEMENTS SURROUNDING THE ALVEOLI OF THE MAMMARY GLAND*

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In connection with the renewed interest concerning the relation of the nerves, hormones and muscles which coordinate the important process of milk "ejection" or "letting down" at the time of milking, it seemed of interest to further investigate the type of cells surrounding the alveoli.

The literature on the subject is reviewed by Turner (1). The earlier investigators quite generally held that muscular cells or epithelial cells with contractile properties (myo-epithelial cells) were present in the subepithelial area of the alveolus. Recent observers have expressed doubt that these cells surround the alveolus directly but have suggested that the smooth muscle or myo-epithelial cells observed surround the capillaries instead.

For many years it has been known that extracts of the posterior lobe of the pituitary called "pituitrin," when injected into lactating animals cause an apparent contraction of the mammary gland and milk becomes available which previously could not be removed. (For review see Turner & Slaughter (2).) As pituitrin is considered to cause contraction of smooth muscles, especially of the uterus and blood vascular system, it seemed logical to assume that the effect of pituitrin upon the mammary gland was to produce a contraction of the muscular elements, forcing down the milk accumulated in the lumina of the alveoli and storage spaces of the duct system.

While the effect of pituitrin upon the mammary gland, uterus and vascular system has been known for a long time, only recently has evidence begun to appear indicating that it may play a role in the contraction of the udder musculature and the "letting down" of milk following the stimulation of the teats at milking time (3, 4, 5). The present concept of the relation of the nerves, posterior pituitary, adrenal and udder has been outlined (6). It should be understood, however, that positive proof is still lacking. As several phases of the problem are being investigated in our laboratory, it seemed desirable to re-examine the cellular structures surrounding the alveoli in order to determine whether muscle cells are present to aid in milk removal.

Experimental technique. The udder of a cow in advanced lactation was obtained. Blocks of tissue were placed in Bouin's fluid within an hour post mortem. The usual procedure of sectioning tissue was followed. Several

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stains were tried but the best results were obtained when the sections were stained first in Delafield's hematoxylin for 5 minutes, washed in water, stained 30 seconds in Van Giesen's stain, washed in water again, then passed rapidly up the graded alcohols ($\frac{1}{2}$ min. in each) cleared and mounted with clarite.

Observations. Van Giesen's stain very clearly differentiated the connective tissue by a brilliant red color. The epithelial and muscle cells were stained yellow to brown with well defined purplish brown nuclei. The smooth muscle cells were differentiated from the epithelial cells by the shape of the nuclei, the former being narrow, oblong structures while the latter were slightly ovoid or round. When the cell walls were observed, the epithelial cells were square or rectangular in shape while the muscle cells were long, narrow and more or less pointed at their ends.

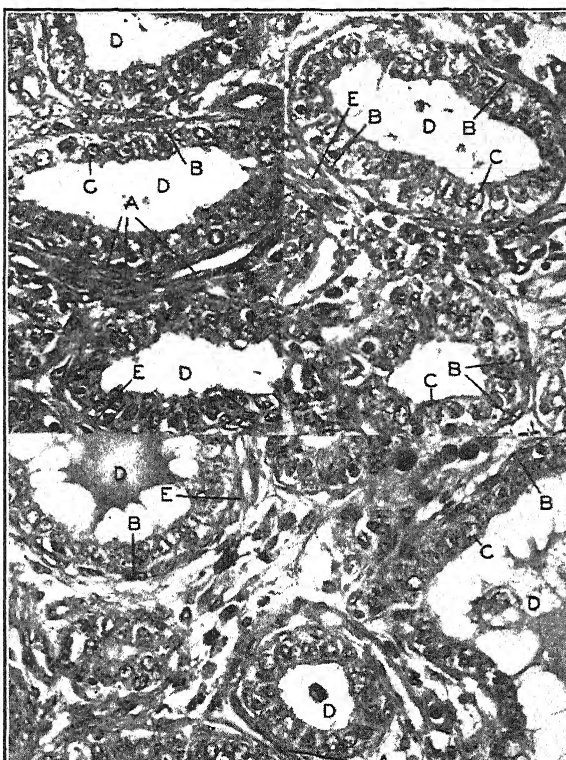

FIG. 1. Sections of mammary gland tissue from a cow in advanced lactation showing cell types of and around the alveoli. A, smooth muscle cells in the interlobular connective tissue; B, smooth muscle cells adjacent to the secretory epithelium; C, secretory epithelial cells; D, lumen of the alveolus; E, interlobular connective tissue fibers. ×375.

Occasional bundles of yellow smooth muscle cells were found scattered among the red connective tissue fibers. Individual isolated smooth muscle cells were also noticed in the interlobular connective tissue. The subalveolar cells were carefully examined. Smooth muscle cells were identified around nearly every alveolus examined (Fig. 1). At times these cells were observed in the interalveolar connective tissue, but frequently they appeared between the connective tissue membrane and the secretory epithelial cells. In no case were the smooth muscle cells observed to form a continuous band around the alveolus. Rather, they seemed to be isolated from each other and were probably of the basket cell type observed by Lenfers (7) and others.

It is difficult to determine definitely whether or not the smooth muscle cells observed directly around the alveolar epithelium are or are not associated with capillaries. However, the capillaries furnishing blood to the secretory epithelium would be of the finest calibre. As a general rule there are neither muscle fibers nor connective tissue in the walls of the true capillaries. It would appear reasonable to believe that these isolated muscle cells around the alveoli contract, thus increasing the pressure on the alveolar lumen contents and forcing the milk into the duct system. The smooth muscles around the ducts seemed to be more numerous and more distinct than those around the alveoli. Upon their contraction there would undoubtedly be a tendency to reduce the length and diameter of the ducts to aid the forward movement of the milk. While no tendency was observed for the muscle cells of the ducts to form in a circular ring or sphincter and it is not believed that muscular sphincters are present at the branching of the ducts, the present method of examination of the tissue does not entirely disprove their presence.

DISCUSSION

The commonplace act of milking sets in motion a complex series of physiological events which are just beginning to be appreciated. The removal of milk from a dairy cow is not as simple as turning a spigot on a barrel and letting the milk flow out. It is believed that the stimulus from the teats, or other sensory stimuli associated with milking, transmits to the pituitary (posterior lobe) a nerve impulse which causes a discharge of pituitrin into the blood stream. Upon reaching the udder, pituitrin causes the contraction of the muscle fibers surrounding the alveoli and ducts and forces the milk into the larger collecting spaces and cistern toward the teat. There is thus an inward squeeze upon every part of the gland which results in aiding the milker to get a greater part of the milk present.

The present study is believed to show that there are muscle cells surrounding the alveoli which upon contraction would aid in evacuating the milk from the lumen, and further that muscle cells line the fine as well as the coarse milk ducts, which upon contraction propels the milk forward to the

teat. Sphincter muscles around the milk ducts were not observed and their presence seems illogical if the above explanation of "letting down" milk is correct. The presence of duct sphincters would call for a complicated control system whereby certain smooth muscles of the ducts would be contracted while the sphincter at the same time must necessarily be relaxed.

SUMMARY

An examination of the udder of a lactating cow by histological means and suitable staining showed the presence of cells beneath the secretory epithelium of the alveoli and in the interlobular spaces which had the appearance and staining properties of smooth muscle fibers. These cells were not observed to form a continuous band around the individual alveoli but were spaced at intervals below the epithelial cells. They are believed to surround the alveoli and to aid in the expulsion of milk from the lumen and not to be associated with blood capillaries. The walls of the duct system also contained similar smooth muscle cells but no tendency was observed for these cells to form muscular sphincters which would, upon contraction, impede the flow of milk.

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THE RELATIONSHIP OF FAT TO QUALITY, AND METHODS OF STANDARDIZING THE FAT CONTENT, IN SWISS CHEESE

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As a result of experimental research conducted in these laboratories and data obtained in factories in the Swiss cheese-producing areas in Wisconsin, Ohio, Idaho, and Pennsylvania, much information has been collected with respect to causes of variations in quality of Swiss cheese. It was shown, in results published previously (3), that the use of milk of poor bacteriological quality (methylene blue reduction time less than 3 hours) resulted in a decrease in the average grade of the cheese, and also that a complete lack of ripeness in the milk was apparently detrimental. In a recent report (4), methods were described which can be used to control the amount of moisture in the green cheese, and it was demonstrated that methods used to reduce the percentage of moisture resulted in a general improvement in quality of cheese.

This paper deals with the relationship between the percentage of fat in dry matter in the cheese and its quality, and with procedures used in predicting the percentage of fat in dry matter in the cured cheese.

RELATIONSHIP OF PERCENTAGE OF FAT IN DRY MATTER TO QUALITY

Factory cheese. The relationship of percentage of fat in dry matter to quality, for 844 cured cheeses in 39 factories, is shown in figure 1. It was found that the proportion of good cheese was largest among those which contained between 45 and 46 per cent fat in dry matter. The results show also that the average quality was better in cheese containing more than 48 per cent fat in dry matter than in cheese containing less than 43 per cent.

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[1] Resigned March 15, 1940.

[2] The work on factory cheese described herein was conducted with the cooperation of the Departments of Dairy Industry of the University of Wisconsin and the Ohio State University.

This was true for data from 19 factories located in Wisconsin, and likewise from 18 factories located in Ohio.

The grades of some of the high-fat cheeses were reduced because the cheeses were soft, weak, or pasty in body, and because they contained "glass" or splits in the curd (glaesler defect). A large proportion of the D grade (grinder) cheeses, in several factories, were soft or weak in body. Results of recent experiments in the laboratory indicate that extreme softness or weakness of body may be a result of the use of milk that contains an abnormally low proportion of casein to other non-fatty solids, and that the presence of a high proportion of fat tends to accentuate the softness.

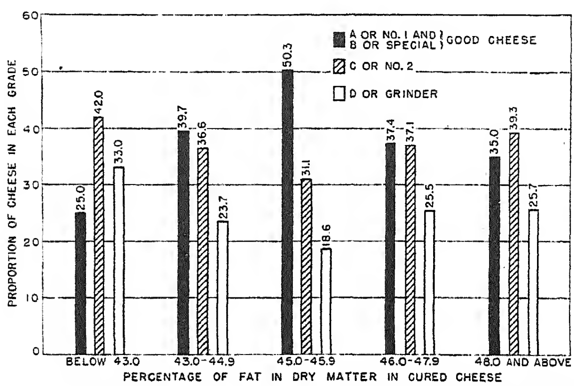

FIG. 1. Relation of percentage of fat in dry matter to quality (844 factory cheeses).

Laboratory cheese. Results were slightly different in the case of smaller, experimental cheese made in the laboratory plant, where the milk used is relatively high in percentage of total solids, and in curd tension, as compared with that used in the cheese-producing areas. Data for 30 pairs of experimental cheese are shown in table 1. These cheeses were the same ones for which moisture and yield data were quoted and discussed in connection with the results shown under variation No. 5 in table 3 of a former publication (4). For one cheese of each pair, the percentage of fat in the milk was reduced below that in the milk for the other by adding a relatively larger quantity of skim milk. Most of these cheeses—particularly those of low fat content—were too firm in body. This property seems to be characteristic of cheese made from high-solids, firm-curd milk, and is overcome to some extent by the incorporation of relatively more fat. The highest quality in factory cheese, on the other hand, was usually found among those which contained less fat in dry matter than was present in the best laboratory cheese.

In the experiments for which data are shown in table 1, better quality was secured in cheese containing more than 48 per cent fat in dry matter than in cheese containing less than 45 per cent. The scores of the low-fat

TABLE 1

Data showing relationship of percentage of fat in Swiss cheese kettle milk to composition and quality of experimental cheese (averages for 30 pairs of 60-lb. cheese, cured 2-2/3 months)

Milk	Cured cheese					
Fat	Fat in dry matter	Total score	Decrease in score			
			Overset	Texture too firm	Glaesler defect (cracks in curd)	Flavor defects
%	%	*points*	*points*	*points*	*points*	*points*
2.9	44.12	69.4	9.4	4.5	0.3	4.6
3.4	48.60	75.0	6.2	1.3	2.6	3.1

cheeses were reduced more than those of the high-fat ones for being overset, for defects in flavor, and for excessively firm body. On the other hand, a few of the high-fat cheeses contained the glaesler or curd-splitting defect, but there was less evidence of it in the low-fat ones. The low-fat cheeses rose more rapidly, and to a greater extent, than the high-fat ones. When cured, they contained an average of 0.55 per cent more moisture than the high-fat ones. Kettle whey from the low-fat cheeses contained an average of 7.23 per cent total solids and 0.50 per cent fat; corresponding data for whey from the high-fat ones were 7.44 and 0.67, respectively.

PREDICTION OF PERCENTAGE OF FAT IN DRY MATTER

Procedures based on analyses of samples of kettle curd. Because of damage done to uncured cheese by taking plug samples, it is very desirable to have a reasonably accurate analytical method for securing and testing samples of kettle curd rather than of uncured or green cheese. Having data on composition of curd, and on its relationship to composition of cured cheese, the cheesemaker can alter the percentage of fat in the milk on the basis of results of curd analyses, and possibly alter the making process, in order to regulate the proportion of fat in cheese made on successive days.

Securing and pressing samples of kettle curd. A photograph of equipment used in securing and preparing samples of kettle curd is shown in figure 2. Two samples of kettle contents were taken immediately before dipping from a point several inches behind the brake where the curd is well mixed by the brake and where the larger particles rise to the surface in greatest numbers. Each sample was taken with a dipper having a bronze screen bottom made of 18-mesh, 28-gauge wire. The cup was $2\frac{5}{8}$ inches in height and $2\frac{1}{2}$ inches in diameter, and the length, including handle, 14 inches. Two dippers were inserted in the revolving kettle contents to a depth corresponding to the length of the handle and were withdrawn with curd contents. Whey was allowed to drain for 2 minutes, after which the curd was loosened from the sides and bottoms by tapping the dippers and curd from both dippers was then placed in one of the metal pressure cylinders. The reason for

taking two small samples instead of one large one was that the probability of securing a representative sample was thus increased.

The cylinders used for pressing the curd samples were $1\frac{7}{8}$ inch in internal diameter and $3\frac{1}{2}$ inches in height. The bottom consisted of a flat metal plate containing about 200 holes each 0.05 inch in diameter, spaced about $\frac{1}{8}$ inch apart. The construction was such that whey drained freely without curd being squeezed out. Each cylinder had 4 legs, each $\frac{1}{4}$ inch long, and was provided with a metal plunger about $3\frac{1}{16}$ inches long, of the proper diameter to fit snugly within the cylinder without binding, and weighing 1000 grams.

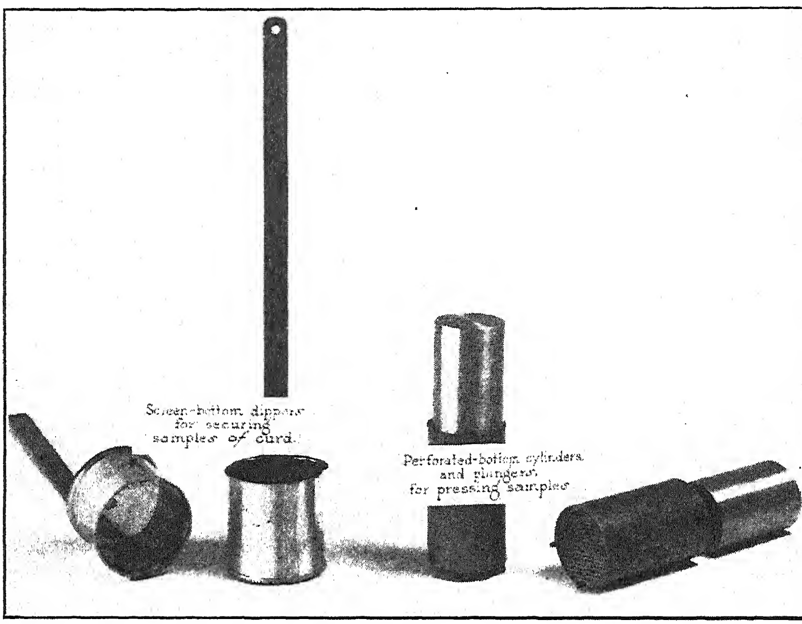

Fig. 2. Equipment for securing and preparing samples of curd from Swiss cheese kettle for analyses.

The plunger was placed upon the sample in the cylinder immediately and allowed to press for 30 minutes. The cylinder was inverted occasionally to allow whey to drain from the upper surface of the sample. The curd, when removed from the cylinder, was either analyzed at once or was wrapped tightly in tin foil, placed in a small, air-tight container, and kept in a refrigerator until analyzed.

For analyses of moisture and fat, each pressed curd sample was sliced vertically through the center, the outer portions were cut off and discarded, and thin slices were removed rapidly from the flat surface. Moisture samples were prepared by cutting the slices rapidly into small pieces with a sharp spatula in a small jar or tumbler; fat samples, by cutting the freshly-prepared slices into strips.

The equipment described above was designed and used in such a way that all curd samples were secured and prepared mechanically in a uniform manner, each one yielding a solid, compact mass which was comparatively similar to the cheese itself in consistency and in composition.

A valuable and exhaustive study of the control of composition in Swiss cheese has been made by Price (2) and his associates. They analyzed a large number of samples of kettle curd and of the corresponding cured cheese and found that the accuracy of prediction of fat in dry matter of cured cheese, based on values obtained in curd analyses, was limited to a range of 1 per cent in 66 per cent of the instances and 2 per cent in 95 per cent of the instances. In their method, referred to herein as the older method, samples of curd were taken from the kettle with a dipper, squeezed by hand to remove as much as possible of the whey, and then analyzed while in a crumbly condition.

Relative accuracy of predictions based on curd analyses. The accuracy of results obtained by the new, pressed-curd method was compared with the accuracy of results obtained by the older, manually-prepared curd method. Percentages of fat in dry matter found by analyzing samples of the cured cheese were taken as the actual or correct values; the amount by which the fat-in-dry-matter value of each sample of curd differed from that of the corresponding sample of cured cheese was calculated. The variations were grouped, according to size, and yielded the data shown in table 2. The results indicate that the use of the new method yielded a greater degree of precision than the use of the older one. For the new method, the average deviation of fat-in-dry-matter values of curd compared with factory-cured cheese was 0.82 per cent, and the standard deviation 1.02 per cent. For the older method, corresponding values were: Average deviation, 1.30 per cent; standard deviation, 1.69 per cent.

TABLE 2

Data showing relative accuracy of results of two different curd-analysis methods of estimating the percentage of fat in dry matter in Swiss cheese

Method of sampling	Samples		Percentage of predicted values falling within specified limits of variation from actual values found in cured cheese				
	Number	Source	0 ± 0.5	0 ± 1.0	0 ± 1.5	0 ± 2.0	0 ± 2.5
			%	%	%	%	%
1	49	Laboratory	49.0	87.8	100.0	....	....
	458	Factory	41.1	69.4	87.5	95.8	98.2
2	136	Factory	32.4	52.2	67.6	80.1	89.0

Method 1 (new method)—Analyses of mechanically-pressed samples of kettle curd.
Method 2 (old method)—Analyses of manually-prepared samples of kettle curd.

Data shown in table 2 indicate also that analytical results obtained in the laboratory were more uniform than those obtained at the numerous factories,

where conditions varied more than in the laboratory and where the most desirable types of analytical equipment, such as an analytical balance and a vacuum oven, were not in all cases available. It seems probable that results of analyses and observations made under factory conditions by careful cheesemakers might not always be as uniform as those reported herein.

Changes in composition during pressing and curing. In analyses of 14 samples of unpressed curd taken from the kettle with a strainer and analyzed without having been pressed, and of the corresponding green cheese, it was found that the average percentage of fat in dry matter increased from 42.5 per cent in the curd at dipping to 46.0 per cent in the cheese. Analyses of samples of the drainage whey showed that the percentage of fat in the whey draining from the cheese gradually decreased from an average of 0.60 per cent at the beginning of pressing to about 0.20 per cent at the time when drainage ended, while the percentage of solids-not-fat in the drainage whey actually increased progressively during pressing. Analyses of the cylinder-pressed curd samples, and of whey draining therefrom, showed very similar results. Koestler (1) found that the percentage of fat in the whey draining from cheese decreased progressively from 0.59 per cent to as little as 0.10 per cent, while the percentage of total dry matter in drainage whey actually increased during pressing. The cause of the increase in percentage of fat in dry matter in cheese during pressing evidently lies in the fact that the loss of solids-not-fat from cheese during drainage is relatively great in comparison with the loss of fat; and therefore the ultimate composition is influenced by the extent of drainage.

TABLE 3

Data showing comparative average composition of curd and factory cured Swiss cheese; curd analyses made by two different methods

Method	Samples		Fat	Moisture	Fat in dry matter
	Number	Source			
			%	%	%
1. New, samples prepared by mechanical pressing	458	Curd	26.07	42.49	45.33
		Cured cheese	27.63	38.84	45.18
2. Old, samples prepared manually	136	Curd	25.47	44.04	45.51
		Cured cheese	27.55	39.30	45.39

The relationship of the composition of the curd, as determined by the new as well as by the old method, with that of the cured cheese is shown in the data in table 3. The percentage of moisture was somewhat higher in the samples of curd than in the cured cheese. The average percentage of moisture found in the curd samples was lower for the new method than for the older one. The average fat-in-dry-matter values for all samples of curd were in general slightly higher than those for all samples of cured cheese.

Results of the present work confirm an observation made by Price (2), who found that when the fat-in-dry-matter value of the curd was unusually low, the fat-in-dry-matter value of the corresponding cheese tended to be slightly higher, but when the fat-in-dry-matter value of the curd was unusually high the fat-in-dry-matter value of the corresponding cheese tended to be slightly lower. We found that among those curd samples whose values were below 43 per cent the average value for the corresponding cured cheese was 0.43 per cent higher, and among the curd samples whose values were above 48 per cent the average value for the corresponding cured cheese was 0.84 per cent lower. The point at which the two trends converged was approximately 44 per cent.

In order to formulate a comparative basis for estimating composition of cured cheese by means of data on composition of kettle curd or of green cheese, analyses were made of samples taken at each of these three stages from 145 laboratory cheeses and 18 factory cheeses. Resulting data are shown in table 4. It was found that the percentage of moisture was in all cases slightly greater in the cylinder-pressed curd samples than in the green cheese; also that the percentage of fat in dry matter was usually slightly less in the curd samples than in the green cheese, but greater than in the cured cheese.

TABLE 4

Data showing average composition of Swiss cheese kettle curd and corresponding cheese, and changes in composition during curing

A. 145 laboratory cheeses each weighing about 60 pounds

	Fat	Moisture	Fat in dry matter	Salt
	%	%	%	%
Kettle curd, pressed	27.02	41.53	46.21	....
Cheese, 1 day old	28.64	38.44	46.52	0.03
Cheese, cured 3 months	29.29	36.45	46.09	0.76
Changes during curing	+ 0.65	− 1.99	− 0.43	+0.73

B. 18 factory cheeses each weighing about 180 pounds

	Fat	Moisture	Fat in dry matter	Salt
	%	%	%	%
Kettle curd, pressed	25.60	42.94	44.87	....
Cheese, 1 day old	27.37	39.13	44.96	0.03
Cheese, cured 2½ months	27.55	38.25	44.62	0.63
Changes in curing	+ 0.18	− 0.88	− 0.34	+0.60

The absolute percentage of fat in the cheese increased consistently during curing. This increase is undoubtedly caused by the fact that shrinkage in curing involves principally a loss of moisture without a proportional loss of fat or of total solids. There was a consistent decrease in percentage of

fat in dry matter during curing—a decrease that was practically accounted for by the increase in dry matter resulting from the absorption of salt. The fact that the average percentage of fat in dry matter decreased by about 0.3 to 0.45 per cent during curing indicates that in order to have some assurance of securing a given average percentage of fat in dry matter in cured cheese it is necessary to have a slightly larger percentage in the kettle curd and in the green cheese.

Calculations based on percentage of fat in kettle milk. Since the fat test of the kettle milk is the only basis of prediction in many factories, it is desirable to determine how accurately the percentage of fat in dry matter in the cheese can be predicted from a knowledge of the percentage of fat in the milk. Therefore, calculations were made by two different methods to show the extent to which the fat-in-dry-matter value of each cheese varied or deviated from the average value, for cheese made from milk of a given fat percentage.

Method 3, based on averages for all cheese sampled: Data for 729 factory cheeses, on which milk fat tests were available, were divided into groups according to the percentage of fat in the standardized kettle milk, and the average percentage of fat in dry matter in cheese in each group was determined. Resulting data are shown in line A, figure 3. The extent to which the fat-in-dry-matter value of each cheese in a group differed from the average value for that group was then calculated. Data showing the distribution of these differences are given in Method 3, table 5.

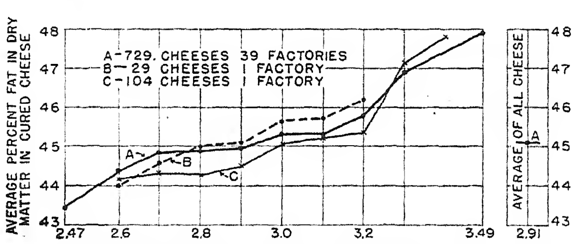

FIG. 3. Data showing average percentage of fat in dry matter in Swiss cheese made from milks of different fat percentages.

Method 4, based on averages for cheese sampled in each of 39 factories, calculated for each factory separately: Data for each factory were first divided into groups according to the percentage of fat in the standardized kettle milk used in each cheese sampled in that factory, and the average percentage of fat in dry matter in cheese in each group was determined for that factory. Resulting data for each of two representative factories are shown in lines B and C, respectively, in figure 3. The extent to which the fat-in-dry-matter value of each cheese sampled in each factory differed from the average value in that factory, for milk of a given fat percentage,

was then calculated. The differences, after being determined for each factory, were then combined and calculated to indicate their distribution, shown in Method 4, table 5.

TABLE 5

Data showing relative accuracy of calculations based on percentage of fat in kettle milk in estimating the percentage of fat in dry matter in Swiss cheese (729 factory cheeses)

Method of calculation	Percentage of predicted values falling within specified limits of variation from average values found in cured cheese				
	0 ± 0.5	0 ± 1.0	0 ± 1.5	0 ± 2.0	0 ± 2.5
	%	%	%	%	%
3	26.2	47.3	62.5	75.8	83.2
4	34.6	60.5	77.8	88.5	94.6

Method 3—Based on averages for all cheese sampled; average deviation, 1.40 per cent; standard deviation, 1.76 per cent.

Method 4—Based on averages for cheese sampled in each of 39 factories, calculated for each factory separately; average deviation, 0.97 per cent; standard deviation, 1.26 per cent.

The results shown in table 5 indicate that standardization based only on the percentage of fat in the cheese milk yielded less precise results for all of the samples as a whole (Method 3) than were obtained in samples in each individual factory considered separately (Method 4). It is to be expected that the number of variables which tend to influence the composition of cheese (such as variations in solids-not-fat content of milk and in fat losses in the making process) is smaller in any one factory than in several factories located in different areas.

The results shown in table 5 indicate also that standardization based only on the percentage of fat in the milk yielded less precise results than were secured by the new, pressed-curd-analysis method (Method 1) for which data are shown in table 2.

The fact that a rather consistent relationship exists between the average percentage of fat in dry matter in the curd and that in the cured cheese (tables 3 and 4) indicates that the percentage of fat in the milk can be used to predict the percentage of fat in dry matter in the curd, and hence is a useful tool in the control of composition.

Our results show, however, that the average percentage of fat in kettle milk required to produce a given average percentage of fat in dry matter in cheese varied seasonally. Tabulations of data on 729 cheeses show that the average milk fat tests which yielded an average of 45 per cent fat in dry matter in the cheese, tabulated by months, were as follows: March, 2.81; April, 2.74; May, 2.75; June, 2.85; July, 2.81; August, 2.82; September, 3.00; October, 3.06; November, 3.08; and December, 3.00. (Data for January and February were not available.) Apparently the percentage of fat in the

standardized milk needs to be increased during the fall and winter months to maintain uniform composition in the cheese.

The results presented above indicate the approximate limits within which it should be possible to regulate, by means of analytical control, the fat in dry matter in Swiss cheese. However, if abnormal conditions (such as the occurrence of mastitis milk) are present or if the analytical facilities available are not adequate for effective control, the results of standardization may not be as uniform as those shown herein.

For efficient standardization it is essential that, in each factory, the milk in each kettle be tested and standardized; it is necessary also that tests be made frequently of the composition of kettle curd and of cured cheese, and that a set of data be prepared in the manner shown in figure 3, to be used in adjusting the percentage of fat in the milk.

Studies of the casein-fat ratio of milk are omitted from this report for the reason that tabulations of such data have not shown, for any one factory, that it is a more accurate basis than the fat test alone for predicting composition of cheese.

SUMMARY

Tabulations of analytical and commercial grading data on 844 factory Swiss cheeses show that the highest average quality was found in cheese containing from 45 to 46 per cent fat in dry matter.

Tabulations of data on 30 pairs of laboratory cheese indicate that when the body of the cheese is relatively firm the presence of a slightly higher proportion of fat tends to improve the quality.

A new method is presented for securing and preparing, in a uniform manner, pressed samples of curd from the Swiss cheese kettle, analyses of which provide a means of estimating the percentage of fat in dry matter in the cheese. By this method, fat in dry matter in the cured cheese was estimated within one per cent in slightly more than two-thirds of the cases.

For efficient standardization it is suggested that, in each factory, the milk in each kettle be tested and standardized; that, for control purposes, pressed samples of kettle curd be secured frequently and analyzed for percentage of fat in dry matter; and that similar analyses be made frequently on samples of cured cheese.

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AN ANALYSIS OF CONTESTANT JUDGMENTS IN THE SCORING OF DAIRY PRODUCTS WITH A STUDY OF SOME FACTORS WHICH MAY AFFECT THEM

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Questions frequently arise in the judging of dairy products relative to the effect of some factors on the efficiency of judging, particularly when a specific number of samples are judged by several groups over an extended period. Inasmuch as 2520 contestant-sample and 8190 contestant-item judgments were involved in the 1940 Students' National Contest in the Judging of Dairy Products, these data seemed to furnish an opportunity for studying what effect such factors as fatigue, order of judging, and quality of product had upon the reliability of judgments.

Sixty-three men comprising 21 teams from state colleges and universities[1] judged 10 samples each of butter, cheese, milk and ice cream. The sample judgments totaled 630 for each product, giving a sum of 2520 for the contest. In arriving at the sample judgment each contestant passed judgment on 40 items for butter (Package allowed perfect score); 30 items for cheese (Finish allowed perfect score); 30 items for milk; and 30 items for ice cream, giving a total item judgment of 2520 for butter, and 1890 each for cheese, milk and ice cream.

In dairy products judging the contestant's grade is a negative grade, being in part the difference between the official score and the contestant's score, and in part, the grade, not exceeding one point per score card item, based upon the contestant's ability to describe the quality as indicated and described by the official judge. Obviously, the contestant with the lower grade has the higher rank in judging ability, inasmuch as his judgment is closer to the official judgment.

Grouping of Contestants. The 63 contestants were divided into four groups so that no two team members were in any one group. Although a definite sequence of numbers was followed in grouping the contestants, the relegation of contestants to groups actually represented random sampling, inasmuch as team members and teams lined up of their own volition for contestant number assignment. In this discussion, the groups will be designated as A, B, C, and D. Groups A, B, and C each had 16 members, whereas group D had 15 members. Numbers 1, 5, 9, etc. were in group A; 2, 6, 10, etc. in Group B; 3, 7, 11, etc. in Group C; and 4, 8, 12, etc. were in Group D.

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[1] Connecticut, Cornell, Illinois, Iowa, Kansas, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Nebraska, New Hampshire, New Jersey, Ohio, Pennsylvania, Purdue, Tennessee, Texas Technological, Vermont, Virginia Polytechnic, and Wisconsin.

Order of Scoring. The order of scoring each product was carried out according to the schedule in table 1.

TABLE 1

The order of scoring and period in which scoring was done by each group

Group	Product assigned to group during			
	1st period	2nd period	3rd period	4th period
A	Cheese	Butter	Ice cream	Milk
B	Butter	Ice cream	Milk	Cheese
C	Ice cream	Milk	Cheese	Butter
D	Milk	Cheese	Butter	Ice cream

Thus, three groups, A, B, and D scored ice cream following butter; three groups, A, B, and C, scored milk after ice cream; three groups, B, C, and D scored cheese after milk; and three groups, A, C, and D scored butter after cheese. The order of products scored by each group was butter, ice cream, milk, cheese, and so on regardless of the product scored first.

Comparative Abilities of the Groups. On the basis of total grades for all products, some differences were noted in the scoring abilities of the various groups, or some factors were operating that influenced the judging. The grades are shown in table 2.

TABLE 2

The scoring abilities of the various groups as shown by the total grades

Group	Total grade per group in the scoring of				
	Butter	Ice cream	Milk	Cheese	All products
A	376.75[2]*	763.45[3]	529.95[4]	580.40[1]	2250.55
B	363.75[1]	790.50[2]	504.25[3]	553.50[4]	2211.50
C	373.50[4]	704.00[1]	533.20[2]	464.50[3]	2075.20
D†	392.80[3]	769.28[4]	533.71[1]	548.00[2]	2243.79

* Numbers 1, 2, 3, 4, indicate the period during which the scoring was done.

† Grades in Group D, composed of 15 contestants, were weighted to compare with those of Groups A, B, and C, composed of 16 members each.

On the basis of the above scores each group would have ranked in the scoring of the several products as shown in table 3.

TABLE 3

The standings of the various groups in the judging of butter, ice cream, milk, and cheese

Group	Standing in the judging of				Total	Rank all products
	Butter	Ice cream	Milk	Cheese		
A	3rd	2nd	2nd	4th	11	3rd
B	1st	4th	1st	3rd	9	2nd
C	2nd	1st	3rd	1st	7	1st
D	4th	3rd	4th	2nd	13	4th

From these scores and rankings it would appear that Groups B and C were slightly superior to the other two groups in judging ability.

Distribution of First 10 Ranking Contestants per Group. The sum of the distributions of the first ten winning individuals in each product according to group presented in table 4 shows that 6 and 15 respectively were in Groups D and C, or 21 out of the possible 40. Thus it would appear that the groups were about equally divided as to abilities except possibly Group C in which were 6 of the 10 ranking individuals in scoring ice cream. Possibly this higher number in one group in the scoring of ice cream may have been due to the period in which the scoring was done.

TABLE 4

Distribution of the ranking 10 individuals in the scoring of each product as to group

Group	Number of first ten men in the scoring of products of each group				Total
	Butter	Ice cream	Milk	Cheese	
A	3	1	4	3	11
B	3	2	2	1	8
C	2	6	3	4	15
D	2	1	1	2	6

The distribution of the same individuals as to the period in which the judging was done is shown in table 5.

TABLE 5

Distribution of the ranking 10 individuals in the scoring of each product according to period in which they judged

Period	Number of first 10 men in the scoring of products as to period in which judging was done				Total
	Butter	Ice cream	Milk	Cheese	
1st	3	6	1	3	13
2nd	3	2	3	2	10
3rd	2	1	2	4	9
4th	2	1	4	1	8

From the above totals, based only on the ranking 10 individuals, it would appear that the best judging was done during the first period, after which the judging was less effective. However, if the number of ranking individuals in the scoring of ice cream per period is disregarded, then the numbers of ranking individuals per period are 7, 8, 8, and 7, respectively. Apparently, from the number of the first 10 ranking individuals in the scoring of ice cream, the first period was conducive to the best judging. In summation, from the data at hand it appears that the groups were fairly well balanced as to judging ability.

The Effect of the Preceding Product on the Efficiency of Scoring. Con-

testants frequently give expression to the thought they would prefer to score one particular product before scoring another, probably on the assumption that it is more difficult to score some one product after having scored another, for instance, scoring milk after previously having scored cheese. The grades, therefore, were compiled in table 6 according to period to note what effect the previous product had on the judging. A study of the data shows that Groups A, D, and C each had higher grades in scoring butter than Group B. Group B started the contest by scoring butter first, whereas, each of the other three groups scored butter in turn after previously having scored cheese. The grades of groups A and C are only slightly higher and are likely not of any significance, but may indicate a trend. It must be borne in mind, however, that during the period of the contest, lasting approximately 5 hours, the butter was exposed to room temperature, so that the condition of the butter, particularly as to body, may not have been the same for each group. This factor may have offset any advantage or disadvantages of the previous product. However, the comparatively high grade of group D scoring butter in the third period would seem unexplainable. It is not unlikely that the lower grade of the group scoring butter first may have been due in part to psychological factors.

In the scoring of ice cream three groups, A, B, and D scored ice cream following butter. Here again the group scoring the product first had the lowest grade, with a rather wide margin below Groups A and B. Inasmuch as the ice cream was held in an electric refrigerator during the contest, the condition of the product, except possibly the sample exposed to show melting quality, would not have changed as did butter.

On the other hand, three groups, A, B, and C, scored milk after having scored ice cream and had equal to or slightly lower grades than Group D, which scored milk first. Since fresh samples of tempered milk were set out for each group, these differences cannot be explained on changes in the sample during the period of the contest. If not the sequence of the scoring, some other factors must have had an influence.

Finally, three groups, B, C, and D, scored cheese after having scored milk previously. From the standpoint of having the organs of the mouth in optimum condition for tasting, and by reason of contrast in intensity of flavor between products, such an arrangement would seem to be ideal for scoring cheese. And apparently it proved to be, for the three groups, instead of having higher grades than the group scoring the product first actually had materially lower grades. The cheese samples, like the butter, were exposed to room temperature during the entire contest which may have been a factor resulting in the lower grades.

The Effect of the Period of Judging on the Efficiency of the Contestant. The total grades per period presented in table 6 indicated that the scoring per period as the contest progressed was fairly constant. However, some

TABLE 6

The total grade per product according to the period of judging

Product	Total grades according to the period of judging				
	First	Second	Third	Fourth	Total†
Butter	363.25 B*	376.75 A	368.25 D (392.80)	373.50 C	1481.75
Ice cream	704.00 C	790.50 B	763.45 A	721.20 D (769.28)	2979.15
Milk	500.35 D‡ (533.71)	533.20 C	504.25 B	529.95 A	2067.75
Cheese	580.40 A	513.75 D (548.00)	464.50 C	553.50 B	2112.15
All products	2148.00	2214.20	2100.45	2178.15	8640.80

* Group.
† Actual total, not weighted.
‡ Numbers in parentheses weighted.

differences were noted. From the sum of grades per period, it is evident that the best judging was done in the third period followed closely by that in the first period, the poorest judging being done in the second and fourth periods, respectively. The poorest judgment in the second period, might be due to psychological factors such as a general "let down" of nervous energy or to lack of concentration resulting from retention of thought of poor judgment in the first period. The poorer judgment in the last period possibly might be explained by fatigue, or by less keenness in checking details, rescoring and general desire to finish. Hardly may the poorer judgments of the second and fourth periods be explained on the change in the products for that factor would have held as well in the third period when the best judging was done.

A Comparison of Average Grades on a Single Score Card Item According to Product and According to the Period of Judging. The average contestant grade per score card item according to group and period of judging is shown in table 7. With the exception of sediment in milk only those items common to several products were included. The average contestant grade will be seen to have varied according to item, product, and period in which the judging was done, thus showing very few trends throughout the contest. However, several data are worthy of special consideration. As a general observation, the grades on flavor criticisms are of about the same value regardless of product, group, or time of judging, with slightly lower values for milk, thus showing that the contestants know the flavor criticism of each product about equally well and probably those of milk best of all. However, the range in grades on flavor score between products would indicate that contestants were at more of a loss in evaluating than in designating the flavor, particularly in scoring flavor of ice cream and milk which apparently caused

more trouble than butter and cheese. It appears that the contestants as a whole had too high an average grade on flavor score of milk considering the low grades on flavor criticism. In other words, it would seem necessary to standardize the score of milk per specific flavor criticism within limits so that the contestant would not be at so great a loss in placing a value upon the flavor encountered.

TABLE 7

The average contestant grade per score card item by groups according to the period of judging

Product	Items	Average contestant grade of a group per sample when judging was done in the following period			
		First	Second	Third	Fourth
Butter	Flavor score	1.14 B	1.18 A	1.23 D	1.03 C
Ice Cream	" "	1.99 C	2.46 B	2.33 A	2.33 D
Milk	" "	2.04 D	2.12 C	1.99 B	1.95 A
Cheese	" "	1.43 A	1.20 D	0.93 C	1.18 B
Butter	Flavor criticism	0.58 B	0.65 A	0.61 D	0.61 C
Ice cream	" "	0.64 C	0.64 B	0.66 A	0.72 D
Milk	" "	0.51 D	0.56 C	0.56 B	0.61 A
Cheese	" "	0.68 A	0.64 D	0.61 C	0.69 B
Butter	Body and texture score	0.19 B	0.20 A	0.22 D	0.30 C
Ice cream	" " " "	0.92 C	1.05 B	0.95 A	0.96 D
Cheese	" " " "	0.90 A	0.90 D	0.72 C	0.89 B
Butter	Body and texture criticism	0.31 B	0.30 A	0.34 D	0.36 C
Ice cream	" " " "	0.58 C	0.58 B	0.57 A	0.61 D
Cheese	" " " "	0.52 A	0.53 D	0.48 C	0.52 B
Milk	Sediment	0.57 D	0.52 C	0.43 B	0.48 A
	Total	13.00	13.53	12.63	13.24

In scoring the body and texture of butter, ice cream, and cheese, the average contestant grade was higher for body and texture score of ice cream and cheese than for butter, since 9, 10 and 2 samples, respectively, were criticized in this respect, and since closer scoring is usually done on body and texture of butter than on ice cream and on cheese. The slight increase of average contestant grade per period in the *score* for body of butter might possibly be explained in changes occurring in the scoring condition of the butter during the contest. Likewise, there was a slight increase in the average contestant grade on body and texture *criticism* from the first to the fourth period of judging, whereas the average grades on criticisms for body and texture of ice cream and cheese remained fairly constant, from one period to another. However, it is extremely doubtful if any significance may be attributed to these increases due to changes in the product. For example, the average contestant grade for sediment in milk, a fixed factor, ranged from 0.57 in the first period to 0.43 in the second period. This difference must be explained from the standpoint of the student, not in changes in the item scored.

Comparison of Average Contestant Grades on a Single Item per Sample per Period. A study of the average contestant grade per sample presented in table 8 and 9 shows considerable variation in the contestant grades on flavor score between samples, particularly on flavor scores of milk and ice cream. The average contestant grade on two samples of milk, numbers 4, which was rancid and feedy, and 8, which was oxidized, were 4.22 and 3.48, respectively; that on the two garlic samples numbers 2 and 6 were 2.89 and 2.75, respectively. Likewise, in the scoring of flavor of ice cream high average grades were encountered with some samples. Apparently, the contestants for the most part were conservative and scored within a narrower range than did the official judges. If the range of official scores is considered, then the highest average grade per point of range was made in the scoring of flavor of cheese with an average of 0.40.

In the scoring of body and textures of butter, cheese and ice cream, comparatively high grades were secured with samples 3 and 9 of butter; samples 8, 9, and 10 of cheese; and samples 5, 6, and particularly 9 of ice cream thus indicating conservatism on the part of the student. It is not beyond possibility, however, that the official judges may have been somewhat severe in scoring these samples.

SUMMARY AND CONCLUSIONS

A critical study was made of 2520 sample judgments in the scoring of butter, cheese, milk and ice cream in the 1940 Students' National Contest in the Judging of Dairy Products, involving 2520 item judgments for butter and 1890 each for cheese, milk and ice cream, a total of 8190. The 63 contestants, assigned to four groups, judged the four products during four periods of 55 minutes each.

The sequence of product judging, namely, butter, ice cream, milk, and cheese was followed throughout. The effect of the previous product on scoring was, therefore, constant except for the starting period.

The two groups scoring butter and ice cream in the first period had slightly lower grades than the groups which scored those products in the second, third, or fourth periods. On the other hand the three groups which scored cheese after having scored milk had a much lower grade than the group which scored cheese first. The best judging of milk was done in the third period.

Excitement and fatigue appeared to be factors of little consequence as indicated by the total scores of the various periods. Slightly lower grades, showing superior judging, were obtained in the third and first periods, respectively; the highest grades, showing poorest judging, being made during the second period.

Higher average grades were made on scoring flavor of milk and ice cream than on scoring flavor of butter and cheese; whereas, little difference was noted between the average grades on criticisms for the four products.

TABLE 8

The average contestant grade on flavor score and on flavor criticism per sample per product

Sample No.	Official flavor		Average contestant grade per sample on	
	Criticism	Score	Score	Criticism
Butter				
1	Coarse	37.0	1.49	0.80
2	Old cream, coarse	35.0	1.00	0.67
3	Old cream, cheesy, neut.	32.0	1.66	0.51
4	....	38.0	1.07	0.68
5	Coarse, old cream	36.5	0.77	0.64
6	Unclean, old cream, neutralized	35.5	0.92	0.46
7	....	38.0	1.13	0.78
8	Coarse	37.0	0.70	0.56
9	Old cream	34.0	0.99	0.59
10	Old cream, nuetralized	33.0	1.75	0.46
		Range 6.0	Avg. 1.15	0.62
		Avg. grade per unit range 0.19		
Cheese				
1	Flat	39.0	0.90	0.67
2	Unclean, acidy	38.5	1.15	0.61
3	Unclean, acidy	38.5	1.02	0.63
4	Unclean, acidy	38.5	0.91	0.67
5	Unclean, acidy	38.0	0.93	0.63
6	Flat	39.0	1.40	0.94
7	Acidy	37.0	1.31	0.52
8	Unclean	36.0	1.76	0.58
9	Unclean	37.0	1.40	0.69
10	Unclean	37.0	1.28	0.65
		Range 3.0	Avg. 1.21	0.66
		Avg. grade per unit range 0.40		
Milk				
1	Sl. cooked	22.0	0.62	0.44
2	Garlic	14.0	2.89	0.53
3	Unclean	19.0	1.90	0.93
4	Rancid, feed	16.0	4.22	0.75
5	Feed	21.0	1.22	0.46
6	Garlic	16.0	2.75	0.70
7	....	23.0	0.74	0.33
8	Oxidized	15.0	3.48	0.22
9	Sl. cooked	22.0	1.58	0.67
10	Sl. cooked	21.5	0.92	0.60
		Range 9.0	Avg. 2.03	0.56
		Avg. grade per unit range 0.22		
Ice Cream				
1	....	45.0	1.50	0.78
2	Lacks fine flavor	44.0	1.21	0.56
3	....	45.0	2.37	0.93
4	Unnatural, metallic, old ingredient	38.0	3.02	0.55
5	Lacks fine flavor	42.5	2.03	0.76
6	Old ingredient, oxidized	39.0	3.05	0.65
7	Unnatural, old ingredient	38.5	3.04	0.56
8	Lacks fine flavor, lacks sugar	43.5	1.00	0.59
9	Old ingredient, storage feed, oxidized	36.0	3.69	0.47
10	Lacks fine flavor	44.5	1.92	0.71

TABLE 9

The average contestant grade on body and texture score and on body and texture criticism per sample per product

Sample No.	Official body and texture		Average contestant grade per sample on	
		Score	Score	Criticism
	Butter			
1	....	25.0	0.09	0.08
2	....	25.0	0.22	0.24
3	Crumbly	24.5	0.43	0.81
4	....	25.0	0.07	0.13
5	....	25.0	0.34	0.48
6	....	25.0	0.14	0.16
7	....	25.0	0.14	0.22
8	....	25.0	0.08	0.16
9	Crumbly	24.5	0.50	0.75
10	....	25.0	0.30	0.29
		Range 0.5	Avg. 0.23	0.33
		Avg. grade per unit range 0.46		
	Cheese			
1	Open	29.0	0.58	0.32
2	Mealy, open	28.5	0.42	0.58
3	Mealy, open	28.0	0.71	0.55
4	Pasty, open	28.0	0.54	0.48
5	Mealy, open	28.0	0.58	0.50
6	Open, sw. c. holes	28.5	0.64	0.46
7	Mealy	28.0	0.78	0.74
8	Gassy, yeast slits, curdy	26.0	1.50	0.65
9	Gassy, yeast slits, curdy	26.0	1.48	0.50
10	Gassy, yeast slits, curdy	26.0	1.30	0.40
		Range 3.0	Avg. 0.85	0.52
		Avg. grade per unit range 0.28		
	Ice Cream			
1	....	24.5	...	....
	or curdy	24.0	0.60	0.53
2	....	24.5	0.71	0.75
3	Curdy	24.0	0.58	0.55
4	....	24.5	....	....
	or curdy	24.0	0.71	0.70
5	Icy, weak	22.0	1.27	0.55
6	Icy, gummy (soggy)	22.0	1.15	0.52
7	Icy	22.0	0.98	0.30
8	....	24.5	0.73	0.84
9	Buttery, gummy (soggy), icy, curdy, does not melt	20.0	2.50	0.60
10	Wheys off, curdy, crumbly, does not melt	23.5	0.52	0.57
		Range 4.5	Avg. 0.97	0.59
		Avg. grade per unit range 0.21		

Contestants appeared to be more conservative in scoring strong off-flavors of milk and ice cream than the official judges.

The change in body and texture of the products during the progress of

the contest as affecting its scorability would seem to be of minor importance, dependent upon the product; changes in cheese being of little consequence; changes in ice cream negligible, except possibly for melting quality; whereas, changes in body of butter were such as to increase slightly the average contestant grades per period of judging.

The distribution of the 10 ranking individuals per period was quite uniform except in the judging of ice cream when 6 of the 10 ranking individuals scored ice cream in the first period.

IN VIVO STUDIES OF HYDROGEN ION CONCENTRATIONS IN THE RUMEN OF THE DAIRY COW

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INTRODUCTION

The rumen of herbivorous animals plays an essential and important role in the digestive process although no digestive juices are secreted in the rumen. Moisture, body heat, and rumen motility provide an excellent environment for the fermentation and maceration of the coarse, bulky, and fibrous material that constitutes the major portion of the herbivore's diet. Cellulose, the main constituent of the crude fiber of plant foods, is utilized by herbivorous animals. Dukes (2) states that bacteria are the chief agencies involved in cellulose digestion and suggests that bacteria ferment cellulose to glucose. For optimal bacterial activity, the hydrogen ion concentration needs to be within a certain range. With possible acid production from carbohydrate fermentation, the pH of rumen ingesta should be influenced by types of feed consumed, which in turn may have a regulatory action on bacterial activity.

Kick and co-workers (3) reported studies with steers on the pH of rumen ingesta. Their determinations varied with rations fed from pH 5.5 to 7.7 with the most alkaline reaction on alfalfa hay alone.

Monroe and Perkins (4) made *in vitro* determinations of the hydrogen ion concentration of rumen ingesta. The pH determinations were made with a Leeds and Northrup portable potentiometer with a saturated calomel half cell and a quinhydrone electrode. Rumen contents were sampled in six different localities and pH readings were taken on uncentrifuged liquid expressed from the ingesta. They gave an average pH value between 6.83 and 7.01 when their animals were fed roughages such as corn and A.I.V silage, and alfalfa hay. A slightly more acid reaction was obtained when the animals were pastured on bluegrass and alfalfa. They reported rumen contents of slaughtered animals to have an average pH of 7.34.

Previous work done at this laboratory showed that some body fluids undergo rapid changes in pH when exposed to air. For this reason it was thought that *in vivo* determinations would be nearer the actual hydrogen ion concentration.

EXPERIMENTAL ANIMALS AND METHODS

The experimental animals used in this study were two Holstein cows with

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permanent rumen fistulas. Both animals were kept in stanchions. Water was available at all times from fountains. One of the animals was used as a nurse cow and the other was milked during the experimental period. The fistulas were not kept closed and a small amount of ingesta was lost when the animals were lying down.[1]

A Beckman pH meter with a glass electrode assembly constructed for such purposes was used in the work. The customary potassium chloride calomel electrode in this assembly is replaced by a silver-silver chloride electrode.

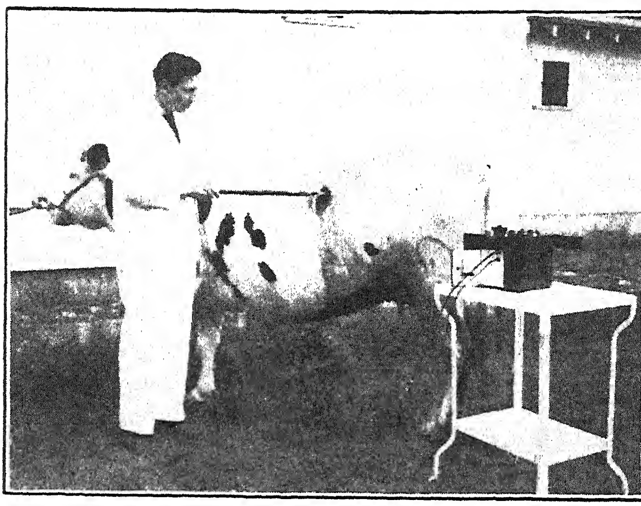

Fig. 1. Illustrates method of making pH readings and equipment used.

This electrode is located on the body of the glass electrode some four inches from the ground portion and connects through the glass to the lead wire provided with a phone tip. The electrode and extension jacket measure 23.5 inches, and are connected to the pH meter by a ten-foot lead.

The electrode was inserted into the rumen, and PH readings were made in six different localities. Readings designated as front were taken in the immediate vicinity of the cardia approximately two inches below the ingesta level. Deep front readings were made with the electrode near the reticulum and a few inches above the floor of the rumen. The middle readings were made in the dorsal sac near the surface and the deep middle readings from the ventral sac an inch or two above the floor of the rumen. Rear and deep

[1] In this study the rumen fistulas were not kept closed because it was found more convenient to take readings in various locations in the rumen. Since submitting this paper for publication, additional studies have been made which show that the pH of the rumen was slightly more acid (0.30) with a closed fistula.

rear readings were made in the dorsal and ventral posterior blind sacs of the rumen respectively.

The experimental period on alfalfa for animal No. 1 was two weeks previous to the corresponding period for animal No. 2. Alfalfa hay and beet pulp experimental periods were simultaneous for the two animals. In order to insure a homogenous ingesta, beet pulp was fed one week prior to the beginning of the experimental period. Readings were made three times a day over a period of five successive days for each animal on each ration. In addition, there were 24-hour periods at the end of the five-day experimental periods in which readings were made every two hours.

RESULTS OBTAINED

Results of pH readings when the animals were on a sole alfalfa hay ration are given in table 1. Means for the various periods represent 30 readings and an overall mean 90 readings. The mean for the 24-hour period is for 72 readings. The readings on animal No. 1 ranged from pH 5.65 to 6.78. The mean pH readings of the 7:30 A.M., 12:30 P.M., and 5:30 P.M. were pH 6.44, 6.22, and 6.13, respectively. The overall mean or mean of all readings for animal No. 1 was pH 6.26. The 24-hour period had a mean pH of 6.29. The range in pH for animal No. 2, when receiving alfalfa hay alone, was 5.85 to 6.85. Means for the various hours are as follows: 7:30 A.M., pH 6.43; 12:30 P.M., pH 6.24; and 5:30 P.M., pH 6.20, with an overall mean of pH 6.29. Mean of the 24-hour period is pH 6.31.

TABLE 1

Comparison of pH means when animals were fed alfalfa hay alone

Experimental animal	pH means of the various periods				
	7:30 A.M.	12:30 P.M.	5:30 P.M.	Overall	24-hour period
No. 1	6.44	6.22	6.13	6.26	6.29
No. 2	6.43	6.24	6.20	6.29	6.31

Table 2 gives the summary of pH readings when the animals received 20 pounds of molasses beet pulp in addition to alfalfa hay ad libitum. For animal No. 1 there was a range in readings from pH 5.29 to 6.54. The mean pH of 7:30 A.M. readings is 6.15; of 12:30 P.M., pH 5.82; and of 5:30 P.M., pH 5.93, with an overall mean of pH 5.97. The 24-hour period mean is pH 6.07.

The range of readings for animal No. 2 was pH 5.55 to 6.59, a mean pH of 6.14 for the 7:30 A.M., 6.01 for the 12:30 P.M., 5.95 for the 5:30 P.M. readings, and an overall mean of pH 6.03. On the readings over the 24-hour period there was a mean pH of 6.07.

From these data it can be seen that the results are consistent. The pH was highest at the 7:30 A.M. readings and lowest at 5:30 P.M. The feeding

TABLE 2

Comparison of pH means when animals were fed alfalfa hay and beet pulp

Experimental animal	pH means of the various periods				
	7:30 A.M.	12:30 P.M.	5:30 P.M.	Overall	24-hour period
No. 1	6.14	6.01	5.95	6.03	6.07
No. 2	6.15	5.82	5.93	5.97	6.07

of beet pulp increased the acidity of the ingesta appreciably. This is evidenced when the pH readings on the beet pulp plus alfalfa hay are compared to the readings of hay alone.

Table 3 presents the comparison of *in vitro* and *in vivo* determinations.

TABLE 3

Comparisons of in vitro and in vivo pH determinations of rumen ingesta

Determination made	Locations from which samples or readings were taken					
	Front	Deep front	Middle	Deep middle	Rear	Deep rear
	Experimental Animal No. 1					
In vitro	6.90	6.55	6.57	6.43	6.50	6.57
In vivo	6.32	6.32	6.32	6.28	6.28	6.25
	Experimental Animal No. 2					
In vitro	6.88	6.49	6.61	6.23	6.17	6.10
In vivo	6.35	6.40	6.10	6.02	5.83	6.16

The *in vitro* pH determinations were made on liquid from ingesta taken from the same parts of the rumen where the *in vivo* readings were made. Samples of ingesta were taken immediately after *in vivo* readings. The *in vitro* determinations were completed within 30 minutes after the samples were taken. The customary potassium chloride calomel electrode was used in making the *in vitro* readings. Table 3 shows that the *in vitro* values were more alkaline than the *in vivo* values in all cases except one.

TABLE 4

Mean pH readings of rumen ingesta in various parts of the rumen

Front	Deep front	Middle	Deep middle	Rear	Deep rear
6.27	6.20	6.05	6.13	6.00	6.13

Mean pH values of rumen ingesta in various parts of the rumen are given in table 4. Means are of readings on both rations and each figure represents 30 pH determinations. The front reading, which was taken near the cardia, has the highest pH value. This is logical, since the 7:30 A.M. and 5:30 P.M. readings were made shortly after the animals were fed. Dukes (2), Kick and associates (3), and Monroe and Perkins (4), assess saliva a pH of 8.00

and over. The contractions of the reticulum keep the ingesta in the cardia region well bathed with liquid ingesta. Rear and middle readings are more acid and were made in the moist ingesta; whereas the deep front, deep middle, and deep rear readings were taken in the more liquid ingesta composed of suspended material, ingested water, and salivary secretions, and were more alkaline.

Figure 2 presents graphically the means of the pH readings at two-hour intervals on animal No. 1. The animal was fed at 7:30 A.M. and 5:30 P.M. The curve representing the alfalfa hay pH readings shows a gradual increase in alkalinity up to 9:00 A.M. after which there is a downward trend until 11:00 A.M. The pH takes an upward trend from then until 5:00 P.M. With minor fluctuations, there is a gradual rise to 1:00 A.M., the high point of the period with pH 6.47. The broken line, which indicates the trend of the mean pH readings on beet pulp, has the highest pH of the day at 7:00 A.M., or thirty minutes before being fed. There is an increase in pH from 1:00 P.M. to 5:00 P.M., a decline to the low point of the day at 9:00 P.M., and a general rise to 3:00 A.M.

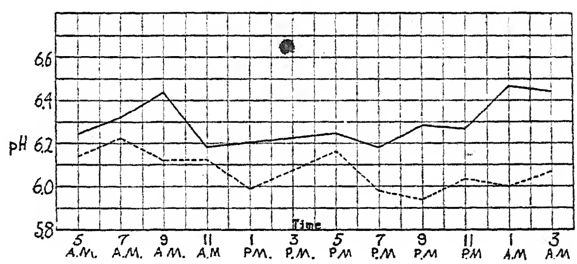

FIG. 2. Means of pH readings taken at two-hour intervals, experimental animal No. 1. Continuous line-alfalfa hay alone, broken line-alfalfa hay and beet pulp.

Figure 3 represents means for animal No. 2. The high point in the continuous line curve is at 9:00 A.M., after which there is a constant decline to 5:00 P.M., an incline to 7:00 P.M., a drop to 9:00 A.M., a slight rise at 11:00 P.M., a lowering at 1:00 A.M., and a rise at 3:00 A.M. The curve for the beet pulp was comparable to the alfalfa curve in that it reached its peak at 9:00 A.M. Another high point occurred at 3:00 P.M., followed by a general trend downward to 11:00 P.M. The curve then takes an upward swing to 3:00 A.M. A study of the graphs shows that the mean pH values on the alfalfa hay and beet pulp ration are without exception lower than the values on the alfalfa alone.

The curves do not follow any consistent pattern with respect to time of day. The fluctuations of these curves are probably influenced by large amounts of saliva entering the rumen during rumination. Schalk and Amadon (5) state that the ox secretes approximately 15 gallons of saliva per day and that 50 per cent of rumen ingesta consists of ingested water and

salivary secretions. They explain that the remasticated bolus is deposited in the anterior dorsal sac of the rumen. Saliva accompanying the bolus would have the opportunity of being mixed with rumen ingesta. Water follows the same path as the bolus and may be a factor in causing pH fluctuations.

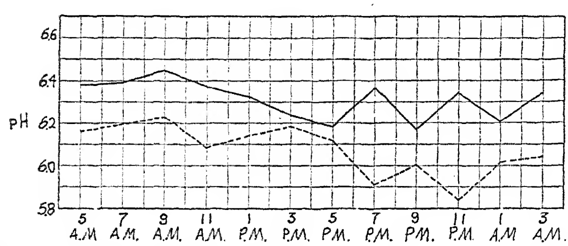

FIG. 3. Means of pH readings taken at two-hour intervals, experimental animal No. 2.

DISCUSSION

Significance of pH in the rumen is as yet unknown. Hydrogen ion concentration may be a factor in bloat in that it may provide optimum conditions for fermentation activities of bacteria and the formation of gases. Dougherty (1) has shown that gas is absorbed from the rumen and that the rate of absorption is increased with an increase of intraruminal pressure. Carbon monoxide was found to be a gas common to the rumen and was capable of producing symptoms of distress in low concentrations when ruminal pressure was increased. Later unpublished work by the same author indicates the hydrogen ion concentrations of the rumen may have a marked influence on the production and the absorption of hydrogen sulfide into the blood stream. The later-mentioned gas was thought to be an important cause of some of the symptoms of acute bloat. Raising or lowering of pH in rumen ingesta may result from the accumulation of products of bacterial activity.

Mean pH values determined in this work are lower than those reported by other workers. These lower values may be due to the fact that pH determinations were made *in vivo*. The more alkaline values for the *in vitro* determinations are possibly due to the loss of CO_2 from the *in vitro* samples.

SUMMARY

Hydrogen ion determinations were made on rumen ingesta, using as experimental subjects two cows with permanent rumen fistulas.

Readings of pH were taken three times a day over a period of five days and at two-hour intervals over a period of 24 hours for two different rations.

A mean hydrogen ion concentration of 6.27 for alfalfa hay alone and 6.00

for alfalfa hay and beet pulp was obtained over a period of five days with three-times-a-day readings.

Over the 24-hour period with two-hour-interval readings, the mean pH for the alfalfa ration is 6.30 and for the alfalfa and beet pulp ration 6.07.

Rumen ingesta fluctuate in hydrogen ion concentration throughout the day but the ingesta are generally more alkaline shortly before and after feeding.

Mean hydrogen ion concentration readings varied from pH 6.27 in the front to pH 6.00 in the rear of the rumen.

Lower pH values were obtained by *in vivo* pH determinations than by *in vitro* determinations.

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THE SIGNIFICANCE OF LIPOLYSIS IN THE CURD TENSION AND RENNET COAGULATION OF MILK. I. THE ROLE OF FAT GLOBULE ADSORPTION "MEMBRANE." II. THE EFFECT OF THE ADDITION OF CERTAIN FAT ACIDS TO MILK

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The relatively low curd tension of fresh sweet cream buttermilk (1, 2) and the failure to clot with rennet of buttermilk obtained from certain creams having other than a natural fat globule adsorption "membrane" (3) have been attributed by Tarassuk and Palmer (3), Palmer and Tarassuk (4) to two possible factors: 1. The curd tension reducing effect of adsorption "membrane" protein as the result of its possible partial denaturation in the process of churning of cream; 2. The interference of certain fat acids with a normal clotting of milk by rennet. The fat acids are liberated by partial hydrolysis of milk fat, which, presumably takes place under certain conditions on the replacement of a natural adsorption "membrane" around the fat globules.

In the present report experimental evidence is submitted to show (a) the extent and significance of lipolysis in curd tension reduction of milk and buttermilk by fat globule "membrane" replacement and (b) the conditions under which certain free fat acids when present in milk will completely inhibit the clotting of milk by rennet.

EXPERIMENTAL

The creams having other than a natural fat globule "membrane" were prepared as described in detail previously (3). Essentially, the procedure was as follows: Fresh, pure butter fat is emulsified in an aqueous solution of the desired emulsifying agent to give the synthetic cream. By diluting this cream with normal fresh skim milk "remade" whole milk is obtained. Centrifugal separation of the "remade" cream on churning produces "remade" buttermilk.

The curd tension, surface tension, pH, . . . values, were determined as described in a previous paper (3). The official method (1936) was employed for the determination of free fatty acids and the values obtained are expressed as acid degree of fat (ml. of 1N NaOH required to neutralize the free acids in 100 grams of fat).

I. THE EFFECT OF LIPOLYSIS ON CURD TENSION AND RENNET COAGULATION OF "REMADE" MILKS

In the previous work (2, 3) dealing with the effect of fat globule "mem-

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brane'' material on the curd tension of ''remade'' milks, the source of milk plasma for such milk was a normal raw skim milk. The employment of raw skim milk inadvertently introduced the possibility of lipolysis as suggested by the abnormally low surface tension and pH value of the ''remade'' buttermilks. In a later report of Palmer and Tarassuk (4) it was shown that when diglycol laurate was added to milk, or used as an emulsifying agent, the hydrolysis of the ester always took place when a raw milk was employed. The hydrolysis of diglycol laurate in milk led, under certain conditions, either to reduced curd tension or to a total inhibition of clotting by rennet. In order to evaluate 1, the effect of adsorption ''membrane'' material and 2, the effect of possible lipolysis on the curd tension of ''remade'' milks, we have repeated independently the essential experiments of Tarassuk and Palmer (3) with the additional modification of using pasteurized skim milk as the source of milk plasma for ''remade'' milks.

Table 1 presents the typical results obtained. In experiment I, the only difference between (a) and (b) parts was that raw skim milk was employed in (a) and identical skim milk pasteurized by holding method was used in (b). The ''remade'' milks in this experiment were made from the same gelatin cream.

Two important things are evident from the examination of the pH and the surface tension values of ''remade'' milks in table 1: 1. Whenever a pasteurized skim milk instead of raw milk was used as the source of milk plasma for ''remade'' milks, there was no significant lipolysis in ''remade'' whole milks or in ''remade'' buttermilks, and 2. In the absence of lipolysis the curd tension reduction in ''remade'' buttermilks was relatively small. No complete inhibition of clotting of buttermilk by rennet was observed in any of these cases. On the other hand, in the experiments which were exactly the same in every respect except that a raw skim milk was used as the source of milk plasma for ''remade'' milks, our data are in complete agreement with the data obtained by Tarassuk and Palmer (3). The ''remade'' buttermilks in this instance, on aging at a low temperature, did not show the slightest visible coagulation on the addition of more rennet than was ordinarily sufficient to produce practically instantaneous clotting. The above ''remade'' buttermilks were characterized by abnormally low pH and surface tension values, suggesting the presence of free fat acids as the result of a rather extensive lipolysis. Though to a much smaller degree than in ''remade'' buttermilk, lipolysis was also evident in ''remade'' whole milks if their source of milk plasma was a raw skim milk. Further evidence of hydrolysis of fat in ''remade'' milks whose milk plasma was a raw skim milk is given in table 3.

It should be noted that in the present experiments, as in the experiments of Tarassuk and Palmer (3), the degree of dispersion of fat globules in synthetic creams was similar to that of natural cream as determined micro-

TABLE 1

Curd tension, pH, surface tension and composition of "remade" milk from gelatin and acid whey powder creams

Experiment No.	Product tested	Curd tension	pH	Surface tension at 20–21° C.	Fat	Solids-not-fat	Remarks
		gm.		*dynes per cm.*	%	%	
I	Gelatin creams						
	(a) *Source of milk plasma: raw skim milk*						
	(1) Original skim	80	6.56	53.9	0.02	9.38	(1) On aging at 7° C. for two hours.
	"Remade" whole	50	6.49	46.2	3.93	7.89	
	"Remade" skim	50	6.51	49.0	0.12	8.38	
	"Remade" buttermilk	35	6.12	38.5	0.30	8.31	
	(2) Original skim	82	....	....	....	....	(2) On aging at 7° C. for 44 hours.
	"Remade" whole	45	....	....	....	....	
	"Remade" skim	54	....	....	....	....	
	"Remade" buttermilk	0	6.05	....	....	....	
	(b) *Source of milk plasma: pasteurized skim milk*						
	(1) "Remade" whole	46	6.63	50.4	4.20	8.13	(1) On aging at 7° C. for two hours.
	"Remade" skim	50	6.60	53.9	0.02	8.58	
	"Remade" buttermilk	36	6.60	49.0	0.80	8.10	
	(2) "Remade" whole	40	....	....	....	....	(2) On aging at 7° C. for 44 hours.
	"Remade" skim	50	....	....	....	....	
	"Remade" buttermilk	32	6.53	....	....	....	
II	Whey powder cream						
	Source of milk plasma: raw skim milk						
	Original skim	68	6.63	53.9	0.04	9.00	On aging at 4° C. for two hours.
	"Remade" whole	46	6.56	45.5	4.00	7.62	
	"Remade" skim	55	6.66	46.2	0.15	7.95	
	"Remade" buttermilk	0	6.19	37.8	0.85	7.58	
III	*Source of milk plasma: pasteurized skim milk*						
	Original skim	40	6.56	53.9	0.03	8.92	On aging at 4° C. for two hours.
	"Remade" whole	26	6.60	49.7	4.10	7.48	
	"Remade" skim	29	6.56	53.9	0.06	7.81	
	"Remade" buttermilk	16	6.70	49.0	1.90	7.48	

scopically. The subsequent lipolysis in "remade" milks, therefore, cannot be attributed to increased surface area of milk fat as has been generally attributed in the case of homogenization of raw milk and cream. The true explanation must lie in the replacement of natural "membrane" material around fat globules. The phenomenon of hydrolysis of milk fat when an unnatural emulsifying agent is substituted for the natural fat globule adsorption "membrane" was pointed out by Tarassuk and Palmer (3). That

such treatment is conducive to lipolysis is also evident from a later report by Krukovsky and Sharp (5) on lipolysis of milk fat emulsified in pasteurized skim milk and diluted to a whole with raw skim milk. These authors chose the term "resurfacing" of fat globules to designate the presence of adsorption layer or fat globules of other composition than a natural one.

In connection with the properties of the fat globule adsorption "membrane" derived from skim milk (skim milk is used as emulsifying agent to make the synthetic cream), the lipolysis takes place in "remade" milks obtained from this cream to about the same extent as in similar milks from gelatin and acid whey powder creams. However, in spite of lipolysis, the coagulation of "remade" buttermilk from "skim milk" cream is normal and curd tension reduction is relatively only slight, as can be seen from table 2. Similar results were obtained by Tarassuk and Palmer (3) when an aqueous solution of skim milk powder was used as an emulsifying agent.

TABLE 2

Curd tension, pH, and surface tension of "remade" milks from "skim milk" and natural fat globule "membrane" creams

Experiment No.	Product tested	Curd tension	pH	Surface tension at 20–21°C.
		gm.		*dynes per cm.*
IV	"Skim milk" cream			
	Source of milk plasma: raw skim milk			
	Original skim	64	6.60	53.9
	"Remade" whole	58	6.49	44.8
	"Remade" skim	61	6.56	49.3
	"Remade" buttermilk	54	5.88	42.0
V	"Natural fat globule 'membrane' " cream			
	(a) *Source of milk plasma: raw skim milk*			
	Original skim	60	6.64	53.9
	"Remade" whole	39	6.54	49.0
	"Remade" skim	42	6.60	51.1
	"Remade" buttermilk	0	6.12	42.3
	(b) *Source of milk plasma: pasteurized skim milk*			
	Original skim	60	6.64	53.9
	"Remade" whole	42	6.62	50.4
	"Remade" skim	46	6.64	52.5
	"Remade" buttermilk	27	6.65	51.0
VI	Natural cream			
	Whole milk (raw)	68	6.58	49.0
	Skim milk (raw)	84	6.59	53.2
	Buttermilk from raw cream	50	6.53	44.0
	Buttermilk from pasteurized cream	48	6.60	46.9
VII	Buttermilk from natural pasteurized cream	48	6.59	47.2
	Buttermilk from natural pasteurized cream + steapsin*	0	5.58	33.6

* On the addition of steapsin to cream, the cream was aged until a distinct rancid flavor was developed, and then churned.

The data in table 2, experiment V, show also that the natural "membrane" material, once removed from the fat globules by churning no longer possesses, to the same extent, the property of protection from lipolysis. In experiment V fresh sweet cream was washed three times, each with four volumes of distilled water at 35° C. The washed cream was churned, and the buttermilk from washed cream, pervaporated to two-thirds of original volume, was used as emulsifying agent in preparation of a synthetic cream. This cream is designated as "natural fat globule 'membrane'" cream. The "remade" milks were made from this cream in the normal way using raw skim milk in (a) and pasteurized skim milk in (b) parts of experiment as the source of milk plasma. This skim milk was obtained from the same lot of whole milk as the washed cream.

For the sake of comparison, in the separate experiment VI, table 2, the typical curd tension, pH and surface tension data of buttermilks obtained by churning a natural fresh, raw cream and the same cream but pasteurized are given.

The additional and direct proof of the phenomenon of total inhibition of clotting of buttermilk by rennet due to lipolysis is demonstrated by the data of the experiment VII, table 2. The buttermilk obtained from cream to which steapsin has been added exhibited exactly the same properties in respect to non-coagulation with rennet, low pH and low surface tension values as the buttermilks from "remade" creams whose milk plasma was a raw skim milk. The fact and the extent of hydrolysis of fat in such creams is also shown by the amount of their free fatty acids; the data are given in table 3. The experiment numbers in this table correspond to the respective experiments of tables 1 and 2.

II. THE EFFECT OF CERTAIN FAT ACIDS ON COAGULATION OF MILK BY RENNET

The phenomenon of inhibition of rennet clot in buttermilks from "remade" creams in which hydrolysis of fat has taken place leads to the question as to the constituent of fat affected and the mechanism involved in the prevention of clotting of buttermilk by rennet. Theoretically, the release of fat acids on hydrolysis of milk fat should cause faster coagulation of milk or buttermilk by rennet and give higher curd tension due to the lowering of pH. And, indeed, that is what happens when fatty acids of lower molecular weight, such as caproic, are added to milk in the amount necessary to lower the pH of milk comparable to the pH of "remade" buttermilks. However, the addition to milk of higher melting point fat acids, such as lauric, myristic or palmitic, inhibits the coagulation of milk by rennet completely when the conditions in respect to the amount added (as judged by the lowering of pH of milk) and aging at low temperature are comparable to those of buttermilk from "remade" creams. The complete inhibition of clotting of milk by

TABLE 3

*Free fatty acids of fat from various "remade" creams**

Experiment No.	Description of fat	Acid degree†
I	Gelatin creams	
	Original fat (before emulsification)	0.67
	(a) Fat from "remade" cream	5.05
	Source of milk plasma: raw skim milk	
	(b) Fat from "remade" cream	0.62
	Source of milk plasma: pasteurized skim milk	
II	Whey powder creams	
	Fat from "remade" cream	5.02
	Source of milk plasma: raw skim milk	
III	Whey powder creams	
	Original fat (before emulsification)	0.78
	Fat from "remade" cream	0.72
	Source of milk plasma: pasteurized skim milk	
IV	Skim milk cream	
	Original fat (before emulsification)	0.61
	Fat from "remade" cream	7.45
	Source of milk plasma: raw skim milk	
V	Natural fat globule "membrane" creams	
	Original fat (before emulsification)	0.71
	(a) Fat from "remade" cream	2.92
	Source of milk plasma: raw skim milk	
	(b) Fat from "remade" cream	0.75
	Source of milk plasma: pasteurized skim milk	
VI	Natural cream	
	Fat from raw cream	0.96
	Fat from pasteurized cream	0.67

* The "remade" creams were about 30 hours old at the time of their churning and isolation of fat for acid degree.

† ml. 1N NaOH/100 gm. fat.

these fat acids[1] is demonstrated by the data of table 4. The addition of lauric (M. Pt. 43.6° C.), myristic (M. Pt. 54° C.) and palmitic (M. Pt. 62.6°–63° C.) acids to milk necessitated melting them and adding them in a liquid state to the milk which was also warmed to the temperature of the melting point of the fat acid added. On the addition of the fat acid to milk the mixture was maintained at the melting-point temperature of the respective fat acid for an additional 20–25 minutes, with frequent vigorous stirring to assure a thorough dispersion of the acid in the milk. After this treatment the milk was cooled in ice cold water to 5–7° C. and aged at 7° C. for at least two hours before the curd tension test was made, the latter being made at 35° C.

The question naturally arises as to the mechanism by which the fat acids

[1] Fatty acids in this experiment were obtained from the following sources: Caproic—from Special Chemical Co., Waukegan, Illinois; lauric, myristic and palmitic from Eastman Kodak Co., and oleic acid was prepared by Dr. J. L. Henderson, by fractional distillation of the methyl esters of olive oil and purification by several crystallizations from acetone at – 50° C. The oleic acid had an iodine number of 88.6.

under consideration inhibit the clotting of milk by rennet and thus reduce the curd tension of milk to zero. The following experimental evidence gives some insight into the phenomenon involved. It was found that on the addition of fat acids to milk in the manner described, it is essential to cool the milk and hold cold for some period of time in order to obtain a zero curd tension. One hour of aging at 7° C. seems to be the minimum aging time necessary. When the milk + 0.3 per cent of lauric acid of experiment II, table 4 was tested immediately upon cooling to 7° C. the clotting of milk by rennet was merely delayed although the curd formed was very weak and soft. The sample of the same milk aged for 1 hour at room temperature gave a normal rennet clot; while the sample aged for 2 hours at 7° C. did not show a trace of visible coagulation on the addition of more than double the amount of rennet necessary for an instantaneous clotting of the original sample of milk.

It can be seen from the data of experiment I, table 4, that the addition of $CaCl_2$ to milk nullifies the inhibitive effect of fat acids in rennet coagulation of milk. The presence in milk of an adequate concentration of Ca^{++} is essential for a normal clotting with rennet. It may be argued then that the inhibitive effect of fat acids on rennet coagulation is due to the formation of highly insoluble calcium salts of these acids. The necessity of cooling and aging in itself speaks against the argument of tying up calcium ions by formation of calcium salts of fat acids as the true explanation. Besides it is very unlikely that an appreciable amount of calcium salts of fat acids will be formed at the pH values experienced. The treatment of milk coupled with the amount and the kind of fat acids necessary in order to obtain a complete inhibition of rennet coagulation suggests an adsorption film of the fat acid on the colloidal complex involved in clotting of milk. The following experimental evidence bears out the film theory and the importance of the physical state of the film in rennet coagulation. As has been stated in the experimental methods the temperature of the milk used for curd tension determination was 35° C. If the milk or "remade" buttermilk, exhibiting a failure to clot because of the hydrolysis of fat or the direct addition of certain fat acids as has been described, is warmed to 50°–55° C., held at that temperature for about ten minutes and then cooled to 35° C., the normal clotting by rennet and the curd tension are restored. The temperature to which the milk has to be warmed previous to cooling to 35° C., at which temperature the rennet is added, depends entirely on the melting point of the fat acid involved in the inhibition of rennet coagulation. Thus, in the samples of milk in which a failure to clot was due to the addition of lauric acid, the warming to the temperature of 42–44° C. for about ten minutes is fully sufficient for a restoration of curd tension. The melting point of lauric acid is 43.6–44° C.

TABLE 4

Effect of addition of certain fat acids to milk on curd tension, pH and surface tension of milk

Experiment No.	Treatment of milk	Curd tension	pH	Surface tension at 20–21° C.
		gm.		*dynes per cm.*
I	Raw skim milk (blank)	69	6.66	53.5
	Raw skim + 0.3% of lauric acid	0	6.19	49.7
	Raw skim + 0.3% of lauric acid + 1 ml. of 5% $CaCl_2 \cdot 2H_2O$	75	....	....
	Raw skim + 0.3% of lauric acid + 2 ml. of 5% $CaCl_2 \cdot 2H_2O$	98	....	....
II	Raw skim milk (blank)	84	6.63	53.9
	Raw skim + 0.3% of lauric acid	0	6.22	49.0
	Pasteurized skim + 0.3% of lauric acid	0	6.20	49.0
	Raw skim + 0.2% of lauric acid	29	6.30	49.0
	Raw skim + 0.1% of lauric acid	80	6.42	49.0
III	Raw skim (blank)	73	6.60	53.5
	Raw skim + 0.3% of caproic acid	92	5.58	49.0
	Raw skim + 0.3% of myristic acid	0	6.07	51.8
	Raw skim + 0.3% of palmitic acid	0	6.14	51.8
	Raw skim + 0.27% of oleic acid	32	6.32	38.5

The data in table 5 illustrate the importance of physical state of fat acids involved in the inhibition of clotting of milk by rennet.

DISCUSSION

Milks and their creams whose natural fat globule adsorption "membrane" material has been replaced by the process of re-emulsification of the milk fat in aqueous solutions of gelatin, whey powder, calcium caseinate, skim milk powder or skim milk itself, exhibit a pronounced lipolysis of fat if their source of milk plasma is raw skim milk. The use of pasteurized skim milk as the source of milk plasma prevents this lipolysis. It is evident then that a normal raw skim milk has an enzyme capable of hydrolyzing milk fat and that a natural fat globule adsorption "membrane" affords some protection from this action.

In the light of the work of Herrington and Krukovsky (6) on lipase action in normal milk and our data on the acid degree of fat from natural raw cream and the same cream pasteurized, it appears that the protection against lipolysis afforded by the natural adsorption "membrane" around fat globules is not absolute. However, in our experience, the extent of lipolysis in normal raw milk[2] is so negligible as to be of no importance from the commercial point of view unless the enzyme is activated. It is interesting to note that all processes of activation of lipase reported in the lit-

[2] The so-called bitter milk of late lactation in which rancidity develops seemingly spontaneously is excluded.

erature, such as, homogenization (7), shaking (8), and temperature manipulation (9), do lead to the disruption and partial replacement or distortion of the natural adsorption layer on fat globules. This fact gives further support to the theory of protection from lipolysis by this natural adsorption layer when it is in the state of orientation on the fat globules as exists in untreated milks. The evidence that the natural fat globule adsorption "membrane" once removed from the fat globules by churning affords less protection against lipolysis suggests the partial denaturation of protein of the fat globule "membrane" as a possible explanation. The Rahn theory of denaturation of surface-active protein material in the process of churning is also supported by the recent work of Clayton (10).

That the phenomenon of curd tension reduction or total inhibition of clotting of buttermilks from "remade" creams by rennet is due largely to hydrolysis of milk fat in "remade" creams has been amply demonstrated by the differences in curd tension obtained in the presence and the absence of hydrolysis of fat in "remade" creams, as well as, by the experiments in which the same phenomenon has been produced by a direct addition of high melting point fat acids to milk or by the addition of steapsin to cream. However, even the very extensive hydrolysis of fat in cream will not lead to a complete inhibition of clotting of buttermilk by rennet in all cases as is shown by the data on "remade" buttermilk from "skim milk" cream in table 2. Since the presence of lower molecular weight fat acids would counteract the curd tension reducing effect of high melting point fat acids it seems safe to assume that the final effect on curd tension would be determined by the amount and the kind of fat acids produced in hydrolysis of fat in a particular cream. This would indicate that the material surrounding fat globules might be a factor in selective hydrolysis by the enzyme.

The clue to the mechanism of inhibition of coagulation of milk by rennet by certain fat acids is found in the fact of the partial or complete restoration of normal properties when the milk is warmed to a sufficiently high temperature to soften or melt the fat acids. On the basis of surface tension data the adsorption of the fat acids by the calcium caseinate complex of milk would be expected. The formation by fat acids of surface films consisting presumably of single layers of oriented molecules is a well-established fact. There is a considerable difference in the packing of oriented molecules in the film depending on the physical state of the fat acid. The packing of molecules of fat acid would be much greater when the acid is in a solid state. This packing of oriented molecules in the film is seemingly great enough when the acid is in a solid state to prevent the action of rennet on calcium caseinate of milk. This explanation is supported by the work of Söhngen, Wieringa and Pasveer (11) in which some experimental evidence is given that the rennin has to be adsorbed on the casein molecule in order to be effective.

TABLE 5

Effect of heat treatment on curd tension of milks whose rennet coagulation is inhibited by hydrolysis of milk fat or by direct addition of certain fat acids

Sample No.	Treatment of milk	Curd tension
		gm.
1	Raw skim (blank). Standard procedure*	81
2	Raw skim + 0.3% of lauric acid. Standard procedure	0
1(a)	Raw skim (blank). Warmed to 44° C., held at 43°–44° C. for 10 minutes and then cooled to 35° C.	79
2(a)	Raw skim + 0.3% of lauric acid. Treated as in 1(a)	71
3	"Remade" skim from gelatin cream. Standard procedure	70
4	"Remade" buttermilk from gelatin cream. Standard procedure	0
3(a)	"Remade" skim from gelatin cream. Warmed to 55° C. and held at 50–55° C. for 10 minutes	67
4(a)	"Remade" buttermilk from gelatin cream. Treated as in 3(a)	42
5	"Remade" skim from whey powder cream. Standard procedure	54
6	"Remade" buttermilk from whey powder cream. Standard procedure	0
5(a)	"Remade" skim from whey powder cream. Treated as in 3(a)	50
6(a)	"Remade" buttermilk from whey powder cream. Treated as in 3(a)	44

* Under the standard procedure rennet is added on warming the milk to 35° C.

CONCLUSIONS

1. A replacement of the natural adsorption "membrane" of the fat globules by other surface-active material in raw milk or cream promotes an extensive lipolysis of the milk fat.

2. The rennet coagulation of buttermilk obtained by churning a natural or synthetic cream in which an extensive hydrolysis of fat has taken place may be completely inhibited. The inhibition is due to the interference of high melting point fat acids with the normal action of rennet.

3. The addition of lauric, myristic or palmitic acid to milk inhibits completely the rennet coagulation if the conditions as to the amount of the acid added and the aging of the milk in cold after the addition of acid, are satisfied.

4. The normal rennet coagulation and curd tension of milk are restored if the physical state of the fat acids involved is changed from the solid to (or near to) the liquid state.

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EFFECT OF HUMIDITY ON MOISTURE CONTENT AND FORMS OF LACTOSE IN DRIED WHEY

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INTRODUCTION

Dried whey contains 60 to 75 per cent of anhydrous lactose in the dry matter, depending largely upon the extent of lactose fermentation prior to drying. If whey is dried by the ordinary spray or roll drying process in the manner customarily used for milk, the lactose does not crystallize but remains in the form of a syrup or glass. Whey dried in this manner is very hygroscopic. At ordinary humidities it will absorb moisture and become sticky, and the lactose will finally crystallize, forming a hard cake which must be broken up. The role of lactose in the caking of dried whey has previously been discussed and demonstrated by Troy and Sharp (4). Several patents have been issued covering various processes for inducing lactose crystallization prior to the complete drying of whey (1).

Holm and Greenbank (2) and Supplee (3) determined the moisture content of dried milk after storage at a series of relative humidities. Troy and Sharp (4) showed that a change of beta to alpha lactose occurred on holding dried milk at a high humidity and that this change was accompanied by the appearance of alpha hydrate crystals in the dried milk. The present paper reports results obtained with dried whey. The lactose content of dried whey is higher than that of dried milk and consequently the changes in the lactose are more strikingly reflected in the behavior of the product when exposed to varying humidities. In one type of non-caking dried whey a large proportion of the lactose is present as crystalline alpha hydrate, in another, as beta anhydride. Much information in regard to the properties of lactose has been gained by a study of methods of drying and the properties of dried whey.

EXPERIMENTAL

Control of humidity. Dried whey samples of 3 grams each were placed in open aluminum dishes 5 centimeters in diameter. The dishes were placed in uniform large desiccators with straight sides. In the bottom of each desiccator were placed two liters of an appropriate solution of sulphuric acid to give the desired relative humidity at 25° C. The sulphuric acid solutions were prepared according to the data given in the International Critical Tables. The dishes were weighed at the end of $\frac{1}{4}$, 1, 3, 5, 15, 21, 34, and 68 days.

Other experiments have shown that several years may be required for

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the complete adjustment of the samples to some of the humidity levels. However, for ordinary practical purposes the adjustment of the moisture content of the samples is fairly complete in one to three weeks.

Types of dried whey. The samples of dried whey studied divided themselves roughly into two groups, depending on whether alpha lactose hydrate or beta anhydride had crystallized to form an appreciable amount of solid phase.

The sample numbers followed by the letter "S" refer to samples prepared by the authors, using small scale manufacturing equipment; otherwise, the samples were from commercial products obtained from various sources.

The process of drying whey in which beta lactose crystallizes as a solid phase was developed by Lavett and is essentially as follows: Whey is first concentrated by evaporation to between 30 and 50 per cent solids. The whey is further concentrated to about 80 or 85 per cent total solids on double atmospheric rolls rotating outward from the pinch. The taffy-like mass removed by the knives drops on the surface of a second pair of double atmospheric rolls placed directly beneath the first pair but turning inward toward the pinch. The mass is continuously seeded with beta crystals adhering to the roll surface and the drying is completed on this second pair of rolls. The dry product removed by the knives is flaky and easily pulverized, and a large part of the lactose is present as beta crystals.

The following processes induce lactose crystallization as the alpha lactose hydrate:

(a) In the Simmons process whey is concentrated to between 60 and 70 per cent solids by evaporation. This concentrated syrup is drawn into crystallizing vats. The cooled mass may be mixed with a portion of whey from a preceding lot, or even with dried whey, in order to seed the mass with alpha hydrate crystals. This mixture is allowed to set for some hours, permitting a considerable amount of alpha lactose hydrate to crystallize. After this setting period the material is broken up into small particles and drying is completed by warmed air.

(b) In the Peebles and Manning process whey is concentrated to between 30 and 50 per cent solids, and sprayed into a conical dryer. The spray drying is so controlled that the whey is not completely dried. The product is then mixed and introduced into a rotating drum, together with a little moisture in the form of steam. The temperature and moisture content are so adjusted as to induce crystallization of the lactose as alpha hydrate. Drying is then completed by air.

(c) Whey is concentrated to 60 per cent solids and is then agitated in a crystallizing vat, preferably with seeding from a previous lot. After a considerable amount of lactose has crystallized and the material has thickened to a very considerable extent, drying and crystallization are completed on

rolls in a vacuum; the rolls are maintained at an interior temperature between 140° and 170° F. In this way, with the completely seeded mass applied to the roll and the temperature maintained well below the so-called inversion point of lactose, drying accompanied by crystallization of the lactose as alpha hydrate is accomplished.

(d) Whey was concentrated by various methods in small plant equipment. Crystallization of lactose as alpha hydrate occurred spontaneously or was induced by seeding. Drying was completed on trays in a drying tunnel.

Results. Figure 1 shows that the adjustment of the moisture content of small samples of dried whey placed at various relative humidities is approximately complete in one to three weeks. Two typical samples of dried whey are illustrated; in one, a considerable amount of the lactose was pres-

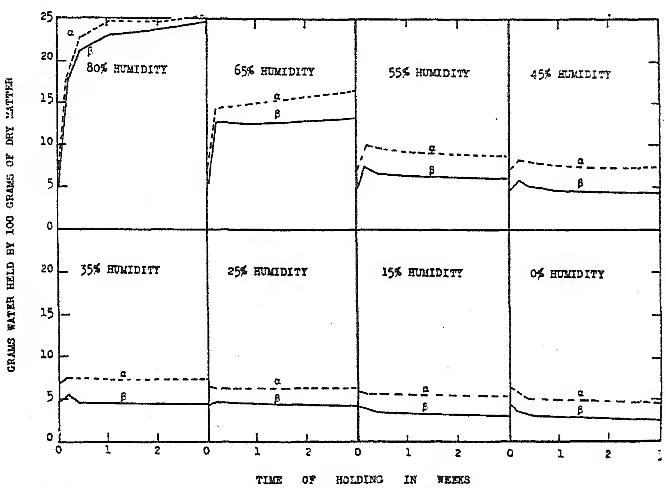

FIG. 1. Water-holding capacity of dried whey at 25° C. at various relative humidities. In one case alpha hydrate, in the other, beta anhydride is present as the solid phase.

ent as crystalline alpha hydrate, in the other, as crystalline beta anhydride. These samples were sealed by the manufacturer and were received in airtight containers. Figure 1 indicates that whey is dried commercially to the extent of being in equilibrium with a relative humidity of approximately 20 to 25 per cent at room temperature.

A number of samples of whey dried by the various methods were tested in order to determine the variations among samples. Some of the data obtained are presented in table 1. It will be observed that the samples roughly divide themselves into two groups. The samples containing beta lactose as a solid phase tended to contain less moisture when exposed to humidities below 65 per cent than samples containing alpha lactose hydrate as a solid phase exposed to the same relative humidities. This would be

TABLE 1

Grams of water held by 100 grams of dry matter in dried wheys kept at various relative humidities for three weeks at 25° C.

Sample No.	Lactose			Relative humidity at 25° C.							
	Total in solids	Proportion of		95%*	80%	65%	55%	45%	35%	25%	15%
		Alpha	Beta								
	%	%	%	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
				Beta lactose solid phase, concentrated, atmospheric drum dried							
28	62.8	21.0	79.0	58.0	25.2	12.7	6.6	5.3	4.8	4.5	3.5
70	71.9	25.5	74.5	50.8	18.8	11.7	4.5	3.4	3.0	4.0	2.9
71	69.0	18.4	81.6	61.6	24.7	14.7	6.1	4.5	4.4	4.2	3.4
36	67.6	19.6	80.4	63.0	24.3	13.2	6.1	4.8	4.4	4.1	3.2
				Alpha lactose solid phase concentrated, seeded, then air dried							
67	69.9	92.0	8.0	62.3	24.2	16.3	8.9	7.7	7.5	6.4	5.6
22	68.1	90.1	9.9	76.0	31.7	15.4	8.0	7.2	6.6	5.5	4.9
26	60.1	97.3	2.7	82.5	28.6	20.6	10.5	8.7	8.1	7.0	6.2
				Alpha lactose solid phase concentrated, spray dried, air dried							
49	68.2	89.7	10.3	52.9	24.5	14.9	9.4	8.5	8.2	7.4	6.7
73	67.7	88.4	11.6	60.3	25.0	15.0	10.0	8.6	8.2	7.6	7.0
				Alpha lactose solid phase concentrated, seeded, vacuum drum dried							
1S	67.3	91.7	8.3	60.6	31.0	....	7.9	....	6.7	6.0	5.0
2S	71.6	55.0	45.0	58.4	30.1	....	7.5	....	6.4	6.1	5.2
				Alpha lactose solid phase, various methods of concentrating and air drying							
10S	63.0	93.2	6.8	74.5	31.3	22.1	11.4	10.9	10.9	10.8	6.6
16S	74.5	91.4	8.6	51.0	21.2	10.4	7.1	6.4	6.2	5.6	5.0
41S	72.4	92.5	7.5	44.4	17.5	12.1	8.7	7.8	7.6	6.8	6.4
47S	70.8	90.0	10.0	48.9	20.2	12.6	8.8	8.1	7.8	7.0	6.2
71S	69.0	88.0	12.0	64.9	27.8	18.9	11.0	9.5	9.1	7.9	7.2

* 7 to 14 days.

expected since the molecule of water which forms part of the alpha hydrate crystal is removed in the moisture determinations, and consequently in table 1 is reported as a part of the moisture content or non-dry matter content. The relative proportions of alpha and beta lactose in the samples given in table 1 represent the composition at the start of the experiment. They were determined from the initial and final (equilibrium) rotations of an extract, clarified with alcoholic mercuric chloride and decolorized with norrit. Total lactose was determined from the final rotation, sample weight and dilution (5).

Figure 2 shows the relationship between relative humidity and moisture-holding power of two typical dried wheys. Large amounts of moisture are

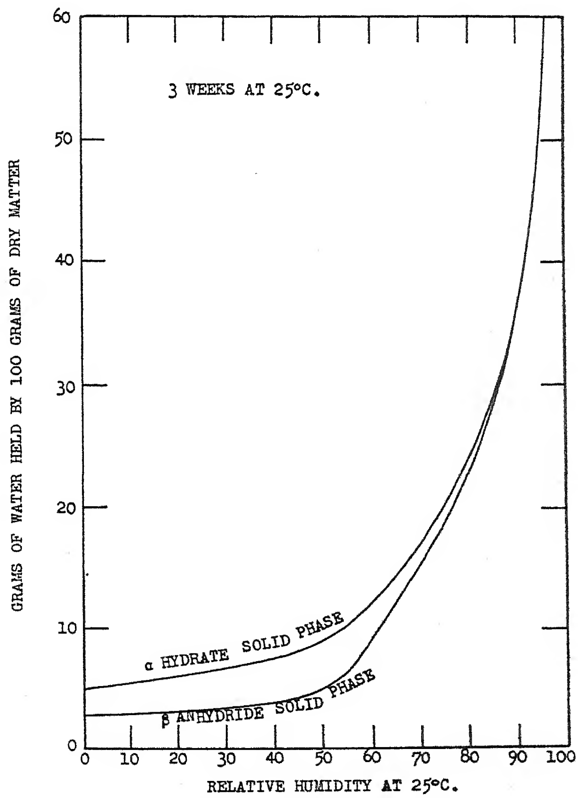

FIG. 2. Effect of relative humidity on the amount of moisture present in dried whey.

not absorbed by stabilized dried whey until the relative humidity exceeds 50 per cent. Table 2 gives the moisture content of a series of additional samples of dried whey when held at different relative humidities for one week and includes the humidity range between 80 and 95 per cent. Samples containing beta lactose as a solid phase contained less water than did sam-

TABLE 2

Grams of water associated with 100 grams of dry matter in wheys held at various relative humidities for one week at 25° C.

Relative humidity	α hydrate solid phase					β lactose solid phase	
	α seeded vac. roll dried		α seeded tray dried			Atm. drum dried	
			Old sample	Fresh samples		Flakes ground	From flakes
	A	B	C	D	E	F	G
%	gm.	gm.	gm.	gm.	gm.	gm.	gm.
95	60.6	58.4	52.2	59.1	52.3	42.3	43.3
90	47.1	45.9	35.8	44.1	42.4	31.7	32.5
85	37.4	35.9	27.4	34.3	33.5	24.7	25.9
80	30.0	30.5	23.5	29.4	28.3	20.6	21.0
55	8.4	8.0	8.5	9.0	8.8	6.2	6.2
35	6.8	6.6	6.9	7.1	6.9	4.4	4.4
25	6.0	6.1	6.2	6.1	6.1	5.1	5.2
15	5.1	5.3	5.9	5.7	5.7	4.3	4.3
0	4.8	4.0	5.0	4.5	4.5	3.1	3.2
0*	3.9	2.6	4.8	3.8	3.8	2.6	2.7

* Over concentrated sulfuric at end of 62 days.

ples containing alpha lactose hydrate as a solid phase. Grinding the sample had little or no effect on moisture absorption. After standing a number of weeks at humidities of 65 per cent and higher there is little difference in moisture content of wheys dried by various methods.

The effect was studied of maintaining the relative humidity constant at 55 per cent but varying the temperature. Results obtained with a series of typical samples are given in table 3. Temperature does not exert a very marked effect upon the equilibrium moisture content of the samples when exposed to a constant humidity. Apparently it does affect the rate of moisture equilibration. The samples maintained at 45° C. had darkened very much by the time the experiment was concluded.

Effect of relative humidity upon the proportions of alpha and beta lactose. The lactose in the various samples of dried whey as freshly prepared by the procedures discussed in this paper is present largely in the crystalline form either as alpha hydrate or as beta anhydride, but a portion, unable to crystallize because of the speed of drying, remains in the glassy state as a mixture of uncrystallized alpha lactose and uncrystallized beta lactose. If the moisture content of the dried whey is maintained at a low value, the lactose remains for long periods of time in an unchanging condition. But if the dried whey is permitted to absorb moisture, as when held at humidities between 30 and 50 per cent and the glass is sufficiently diluted to permit the movement of molecules, the particular solid form of lactose present continues to crystallize slowly, be it alpha hydrate or beta anhydride. Mutarotation takes place simultaneously to compensate for the corresponding

TABLE 3

Grams of water held by 100 grams of dry matter in dried whey after various periods of holding at 55 per cent relative humidity at 2, 25, and 45° C.

Number of days held	Beta type				Alpha type									
	28	70	71	36	67	22	26	49	73	10S	16S	41S	47S	71S
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
45° C. (113° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	7.4	4.7	7.1	6.9	9.7	8.7	11.5	10.6	10.9	12.9	7.3	8.7	9.0	11.6
3	7.4	4.5	6.7	6.6	9.2	8.5	11.2	10.2	10.8	12.5	7.2	8.7	8.8	11.5
7	6.4	4.2	6.2	6.2	8.7	8.0	11.0	9.7	10.0	11.7	7.0	8.4	8.5	11.2
14	5.9	4.4	5.8	5.9	8.4	7.8	10.2	9.3	9.6	11.5	7.0	8.3	8.3	10.9
21	5.5	4.6	5.6	5.6	8.3	7.7	9.8	8.8	9.2	10.5	6.9	8.3	8.3	10.7
25° C. (77° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	7.8	5.2	7.3	7.4	9.9	9.0	11.1	10.6	10.4	12.2	7.5	9.1	9.6	11.6
3	7.4	4.8	7.2	6.9	9.5	8.8	11.0	10.3	10.4	12.1	7.3	8.9	9.3	11.4
7	7.0	4.5	6.4	6.5	9.2	8.0	10.7	10.0	10.1	11.6	7.1	8.7	8.9	11.1
14	6.7	4.4	6.2	6.2	9.0	8.0	10.5	9.5	10.0	11.4	7.0	8.6	8.7	11.0
21	6.6	4.5	6.1	6.1	8.9	8.0	10.5	9.4	10.0	11.4	7.1	8.7	8.8	11.0
2° C. (34° F.)														
0	4.5	3.6	4.5	4.3	6.7	5.4	7.1	7.6	10.8	7.8	5.1	7.1	7.1	8.1
1	8.0	6.1	7.2	7.7	8.4	7.9	9.4	9.4	9.4	10.0	7.2	8.3	8.7	10.0
3	8.2	7.2	7.6	8.0	8.9	8.5	10.0	9.7	9.4	11.0	7.4	8.5	9.1	10.6
7	7.5	5.5	6.9	7.2	9.1	8.4	10.2	9.7	9.4	11.1	7.2	8.5	9.1	10.7
14	7.2	4.6	6.4	6.7	9.0	8.4	10.3	9.6	9.4	11.1	7.2	8.5	9.1	10.6
21	7.0	4.5	6.2	6.5	9.0	8.5	10.3	9.6	9.4	11.1	7.2	8.5	9.1	10.5

depletion in the glass, a disturbance of equilibrium. Evidence that this change has occurred is found in the altered relative proportions of beta and alpha lactose as well as in the absorption and subsequent liberation of moisture at constant relative humidity.

TABLE 4

Percentage of total lactose in the alpha form after holding 3 weeks at 25° C. at various relative humidities

Sample No.	Relative humidity at 25° C.						
	95*	80	65	55	45	35	25
	%	%	%	%	%	%	%
Beta solid phase, concentrated, atmospheric drum dried							
28	86.5	95.4	41.3	7.0	7.7	9.1	14.6
70	86.0	95.1	93.8	12.7	11.5	12.9	19.6
71	84.3	94.3	81.2	7.2	7.2	10.3	14.0
36	84.8	92.4	46.0	6.0	9.4	8.0	13.3
Alpha solid phase, concentrated, seeded, air dried							
67	72.9†	88.8†	92.0	94.4	93.4	90.5	90.5
22	79.0	93.5	95.5	93.2	90.5	88.2	87.0
26	75.8	83.3†	92.9	97.2	95.9	91.9	90.5
Alpha solid phase, concentrated, spray dried, air dried							
49	85.3	92.4	93.4	93.4	91.0	88.5	88.9
73	82.4	89.1	91.8	91.0	84.5	87.7	88.8
Alpha solid phase, various methods of concentrating and air drying							
10S	71.4	91.4†	92.0	98.2	93.0	92.1	87.4
16S	86.0	91.6†	93.9	94.9	90.9	91.5	87.2
41S	89.4	94.6	92.6	91.7	90.9	91.6	87.9
47S	84.2	94.6	93.2	90.6	90.4	87.7	86.1
71S	85.2	92.5	91.3	89.0	90.9	88.4	88.0

* Held 2 weeks.
† Mold.

Table 4 gives in per cent that fraction of the total lactose existing as the alpha form in the samples, at the end of the experiment presented in table 1. Table 4 shows that at about 50 per cent relative humidity and 25° C. additional alpha hydrate crystallized in those samples containing alpha hydrate as the solid phase, and additional beta anhydrate crystallized in those samples containing beta lactose as solid phase. The proportions of alpha and beta lactose in the dried whey after holding at 50 per cent humidity were altered. They were altered in a direction corresponding either to crystallization of alpha hydrate, or of beta anhydride, the direction depending on the solid form present. This is indicated by a comparison with the original product or the product held at 25 per cent humidity for the same length of time.

The original analyses for some of the samples do not agree very well with those obtained from corresponding aliquots held at 25 per cent relative humidity. The direction of the discrepancy suggests that some of the samples had accidentally become exposed to moisture in the interval between the original analysis and placing aliquots in the humidity jars.

At higher humidities of 65 and 80 the dilution of the dried whey containing crystals of the beta type was such as to permit alpha lactose hydrate crystals to form. Since alpha lactose hydrate is far less soluble at 25° C. than is beta lactose, conversion of the solid beta lactose anhydride proceeded, through solution, to alpha lactose hydrate. At 95 per cent humidity the amount of moisture held by the whey was so great that an appreciable amount of lactose dissolved in the water. Therefore, the amount in the form of alpha lactose decreased again because crystalline alpha hydrate dissolved to form a relatively large amount of equilibrium solution. Table 5 shows that at 55 per cent relative humidity additional crystallization of lactose occurred more rapidly the higher the temperature, even though the results may appear to be a little uncertain at 45° C. because of difficulty in

TABLE 5

Percentages of total lactose in the alpha form after holding for 3 weeks at 55 per cent relative humidity at various temperatures

Sample No.	Relative humidity 55 per cent		
	2° C. 34° F.	25° C. 77° F.	45° C. 113° F.
	%	%	%
Beta solid phase, concentrated, atmospheric drum dried			
28	11.0	7.0	5.8
70	15.4	12.7	6.4
71	10.0	7.2	4.4
36	11.4	6.0	6.8
Alpha solid phase, concentrated, seeded, air dried			
67	91.3	94.4	92.4
22	88.4	93.2	96.8
26	93.1	97.2	96.2
Alpha solid phase, concentrated, spray dried, air dried			
49	89.2	93.4	95.5
73	86.8	91.0	95.6
Alpha solid phase, various methods of concentrating and air drying			
10S	91.9	98.2	99.6
16S	90.6	94.9	92.4
41S	91.1	91.7	94.6
47S	88.6	90.6	95.0
71S	87.0	89.0	92.4

analyzing some of the dark products formed at this temperature. Faster crystallization at higher temperatures occurred in both alpha and beta lactose wheys. This variation in crystallization accounts for some of the variations in moisture content of the whey in table 3.

When either dried whey or dried milk containing lactose in the form of glass is exposed to a relative humidity in the neighborhood of 50 per cent, the product at first takes up moisture to dilute the glass, and then as lactose crystallizes and the vapor tension increases moisture is released again. Consequently, there is observed an increase followed by a decrease in moisture content of the samples (3, 4). Table 3 shows that this change occurs more rapidly at higher temperatures, and table 5 presents the evidence of crystallization as reflected by the change in relative proportions of the forms of lactose.

Effect of amount of lactose in the dry matter. In the course of this work a considerable number of samples containing varying percentages of lactose in the dry matter were allowed to come to equilibrium with atmospheres of several constant relative humidities. Figure 3 shows that the lower the percentage of lactose in the dry matter, the higher the moisture content of the dried whey when exposed to an atmosphere of constant relative humidity. This figure also indicates that differences between moisture contents of dried wheys exposed to constant relative humidity are greater, the higher the relative humidity. At lower humidities samples containing beta lactose as a solid phase arrange themselves into one group, and those containing alpha lactose hydrate as a solid phase arrange themselves into another group having higher moisture content. This difference between the two types of samples is maintained up to relative humidity in the neighborhood of 65 per cent. Above this relative humidity there is only one group for the reason that at the high relative humidities the solid beta lactose disappears and is converted to solid alpha lactose hydrate. Thus all samples are very much alike in solid lactose phase.

Commercially, the main variation in lactose content of the dry matter results from varying degrees of fermentation of the sugar prior to drying. Wheys produced by mineral acid precipitation or rennet coagulation of the casein in fresh skim milk tend to be relatively high in lactose whereas cheese wheys tend to be low because of the fermentation of the lactose prior to drying. Lactic acid is the main fermentation product and we would expect the hygroscopic properties of such dried whey to be more pronounced because one molecule of osmotically active lactose would be converted into four molecules of osmotically active lactic acid. This probably accounts for the greater hygroscopicity of the dried wheys containing the lower percentages of lactose in the dry matter.

SUMMARY

1. Dried wheys in which crystalline beta lactose is present as a solid phase contain less water when in equilibrium with an atmosphere of con-

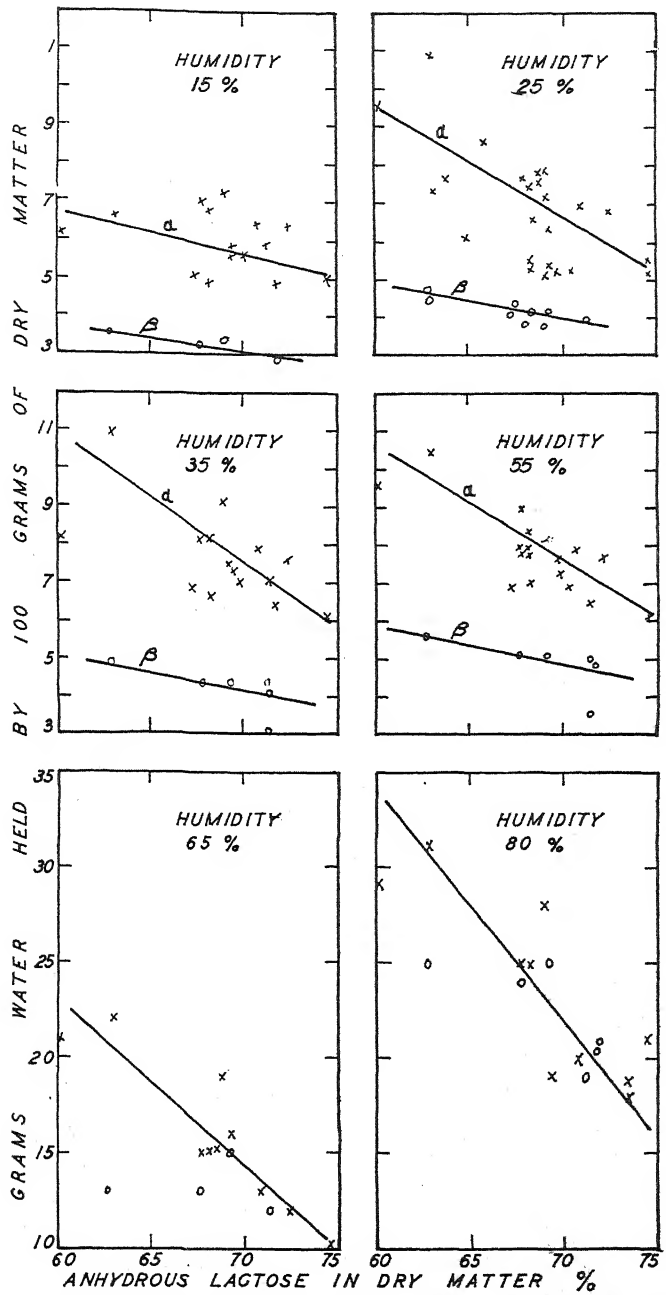

FIG. 3. The effect of percentage of lactose in the dry matter upon the moisture content of dried whey when exposed to various atmospheres of constant relative humidity at 25° C.

stant relative humidity below 65 per cent than do dried wheys in which alpha hydrate is the solid phase. The difference is largely accounted for by the molecule of water of crystallization present in the alpha hydrate crystals.

2. Stabilized dried wheys do not absorb excessive amounts of water until the relative humidity exceeds 40–50 per cent.

3. Temperature exerts no marked effect upon the equilibrium moisture content of samples exposed to constant relative humidity. The equilibrium is attained more rapidly at the higher temperatures.

4. If an appreciable amount of lactose in the glass state is present at relative humidities between 30 to 50 per cent the whey will first absorb and then reject water. This process is accompanied by the crystallization of the solid form of lactose present. In this way crystallization of beta lactose at room temperatures may occur.

5. At relative humidities of 65 per cent and above the crystalline beta lactose in dried wheys undergoes conversion to crystalline alpha hydrate.

6. The lower the percentage of lactose in the dry matter the greater the equilibrium moisture content of the dried whey at constant relative humidity.

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THE EFFECT OF CURD TENSION OF MILK ON THE UTILIZATION OF ADDED VITAMIN D

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The fortification of milk with vitamin D concentrates has been practiced for some time. At first no question arose as to the possibility of the effectiveness of the added vitamin D varying with the type of milk fortified since practically all milk so treated was of the ordinary pasteurized variety. After the introduction of soft curd and homogenized milk and the presentation of some evidence that digestibility or calcium assimilation or both might be favorably affected by such processing (1–5), the effectiveness of vitamin D additions to such milk seemed worthy of consideration.

A supply of mixed milk was divided into three portions and prepared as follows: 1, untreated; 2, homogenized at 2500 pounds pressure and; 3, mineral modified (6, 7).[1] All three samples were then pasteurized at 143° F. for 30 minutes. A batch of natural soft curd milk was collected from selected cows in the Ohio State University herd and similarly pasteurized. Similar batches of milks were prepared at weekly intervals.

After each batch of milk was prepared the curd tension was determined by the tentative method adopted by the Committee on Curd Tension Measurements at the June, 1938, meeting of the American Dairy Science Association. Each batch was then fortified to the extent of 400 U.S.P. units of D per quart by adding a commercial vitamin D concentrate (Cream Vitex).

The samples thus prepared were fed to rats according to the official method for determining vitamin D by the line-test procedure and also by the prophylactic procedure which uses bone ash as a criterion.

RESULTS

The curd tension measurements are summarized in table 1. Both surface and below surface readings were made but only the maximum or surface measurement is recorded, in keeping with the Curd Tension Committee's recommendation. Each value listed is the average of two or more readings. It will be seen from table 1 that the curd tension variation was considerable, from 0.0 grams in the mineral modified milk to 54.9 grams in the normal pasteurized milk. It is also interesting to note that homogenization at 2500 pounds pressure reduced the curd tension by 61.5 per cent.

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[1] Base-exchange method; prepared through the courtesy of M. and R. Dietetic Laboratories, Columbus, Ohio.

TABLE 1

Curd tension of normal pasteurized milk, homogenized milk, mineral modified soft-curd milk, and natural soft-curd milk

Batch	Normal pasteurized milk	Homogenized milk	Mineral modified soft curd milk	Natural soft curd milk
	gm.	*gm.*	*gm.*	*gm.*
1	51.5	21.0	0	35.5
2	43.5	15.0	0	46.0
3	50.0	27.0	0	29.0
4	62.5	27.0	0	35.0
5	69.0	22.0	0	34.5
6	56.5	17.0	0	25.5
7	50.0	23.5	0	32.0
Av.	54.9	21.8	0	33.9

In the curative trial (line test procedure), the results of which are re-reported in table 2, 6.0 cc. of each kind of milk were fed over a period of 3 days. The rats were slaughtered on the eighth day, they having had meanwhile free access to the basal rickets-producing diet. One group of rats received 26 milligrams of reference cod liver oil (2.5 units), an amount of vitamin D calculated to equal that in 6.0 cc. of milk. That the responses from the milks were greater than from the reference oil may have been due to the fact that the milks contained not only the added 400 units per quart but the vitamin D originally present, even though this amount was not sufficient to produce a response when 6.0 cc. each of the normal unfortified and normal soft curd unfortified milks were fed.

From the healing responses obtained when equal amounts of the various fortified milks were fed (table 2) it is apparent that there was no difference in the effectiveness of the added vitamin D. The slight differences in the average line test values are too small to be of significance.

TABLE 2

Comparison of line test responses (healing) obtained by fortifying different kinds of milk with 400 U.S.P. units of vitamin D per quart

Material fed	Total amount fed	No. of rats	Line test* response
Normal milk, fortified	6.0 cc	10	1.70
Homogenized milk, fortified	6.0 cc.	10	1.60
Mineral modified milk, fortified	6.0 cc.	9	1.72
Natural soft curd milk, fortified	6.0 cc.	9	1.78
Reference cod liver oil	2.5 U.S.P. units	10	1.45
Normal milk, unfortified	6.0 cc.	5	0.0
Normal soft curd milk, unfortified	6.0 cc.	3	0.0

* The numerical line test responses were obtained by assigning the following values to degrees of healing:

− = 0.0	++ = 2.0
+− = 0.5	+++− = 2.5
+ = 1.0	+++ = 3.0
++− = 1.5	

In the prophylactic trial the rats in all but two groups were fed 2.7 cc. daily for four weeks of one of the fortified milks while having free access to the basal rickets-producing diet. The rats in one of the remaining groups each received daily 1.1 U.S.P. units of vitamin D from reference cod liver oil. This amount of vitamin D was calculated to be equivalent to the amount contained in 2.7 cc. of 400-unit milk. The final group of rats received only the basal diet.

At the end of 4 weeks the rats were killed. The femurs were removed and the bone ash determined on a fat free, moisture free basis.

TABLE 3

Comparison of calcification (bone ash) obtained by fortifying different kinds of milk with 400 U.S.P. units of vitamin D per quart (4-week prophylactic trial)

Material fed	Amount fed daily	No. of rats	Gain in weight	Bone ash
			gm.	%
Normal milk, fortified	2.7 cc.	10	41.5	46.17
Homogenized milk, fortified	2.7 cc.	10	41.3	46.37
Mineral modified milk, fortified	2.7 cc.	10	41.3	45.68
Natural soft curd milk, fortified	2.7 cc.	10	39.6	45.99
Reference cod liver oil	1.1 U.S.P. units	9	18.4	43.02
Basal diet only	ad lib	7	19.1	27.87

The results of the prophylactic trial (table 3) substantiate those obtained by the curative procedure in that no significant differences were found between the bone ash values of the groups fed the fortified milks.

DISCUSSION

In keeping with previous evidence, the data in table 1 demonstrate the curd tension-reducing effect of mineral modifying (base-exchanging) and homogenizing milk. If certain beneficial effects, such as greater digestibility, lower stomach-emptying time and increased efficiency of calcium utilization result because of such processing, as has been claimed, it might be inferred that each individual constituent was likewise benefited. That added vitamin D showed no difference in utilization may have been due to the fact that such addition did not become an intimate part of the colloid but remained a separate phase.

Although the vitamin D additions were made after the milks had been processed, a survey of a number of large plants showed that as many plants made the vitamin D addition after homogenizing as before. In preparing mineral-modified milk the usual procedure is to process over the zeolite bed before the vitamin D addition is made. In the case of natural soft curd milk the addition would, of course, be made as in other unprocessed milks

It would seem, therefore, that no claim can legitimately be made as to any merit for fortifying soft curd milks with vitamin D greater than that existing for similar additions to milk of normal curd tension.

It might be presumed that the curative trial was a measure of vitamin D *per se* and that the prophylactic trial measured not only vitamin D but calcification. It is known that lactose improves calcium utilization (8) and the higher bone ash values in the fortified milk groups than in the reference oil groups are probably accounted for by this plus the effect of the vitamin D originally present. Furthermore, a normal bone ash can be obtained by feeding an adequate amount of vitamin D in oil. This would seem to obviate the argument that in the prophylactic trial even though the mineral modified milk contained about 20 per cent less calcium than the other milks (6) calcification was just as great and therefore the calcium was more efficiently used.

SUMMARY AND CONCLUSIONS

Normal milk, homogenized milk and mineral modified soft curd milk, all from the same source, and natural soft curd milk were pasteurized and fortified to the extent of 400 U.S.P. units of vitamin D per quart. These milks varied in curd tension from 0.0 grams for the mineral modified milk to 54.9 grams for the normal milk. Bioassays of these fortified milks by the line test procedure resulted in the same degree of healing when equal amounts of each were fed. In a prophylactic trial equal intakes of the fortified milks resulted in almost identical bone ash values.

It is concluded that the effectiveness of added vitamin D is not influenced by the curd tension of milk.

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EFFECTS WHICH SELECTION OF DAMS MAY HAVE ON SIRE INDEXES*

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INTRODUCTION

Several studies (1, 2, 3, 4, 5, 8) have shown that when the mates of a bull are divided into a high group and a low group on the basis of their own records, the average difference between the daughters of the two groups is less than half the difference between the two groups of dams. For example in Edwards' study (3) where the mates of each of 23 bulls were divided into a high half and a low half on the basis of the mate's own milk record, the low half averaged 7,513 and the high half 10,369, a difference of 2,856 pounds. But the daughters of these two groups averaged 7,835 and 8,427, a difference of only 592 pounds, which is barely one-fifth the difference between the two groups of dams.

If offspring were always mid-way between the phenotypes of their parents regardless of how the parents were selected, or if the offspring deviated individually from that only in a random way, the average difference between two groups of daughters by the same sire would tend to be half as large as the average difference between their dams (9). It is clear that the difference between the daughters is not this large when the dams are separated into high and low groups on the basis of the very same past records which are then used to represent those dams. What does this reveal about the inheritance of differences in milk and fat production? Does it disprove, as some have inferred, the validity of the widely used sire index which sets the breeding value of the sire as equal to twice the average of his daughters minus the average of his mates? What precautions should be taken to discount the effects of selection of dams when using indexes or other data to guide breeding choices in an actual dairy population?

In a qualitative way the answers to those questions are partly known already from the simple statistical principles for estimating a cow's real ability[1] from records of her past production. That a cow's record and her real ability are not always identical is well known from the fact that the intra-herd correlation between different single records made by the same cow, when standardized for age and times milked per day, has in most studies been something of the order of +.3 to +.4, rarely going as high as

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[1] By "real ability" is meant what the cow is most apt to produce in any future lactation when she is kept under conditions *intended to be* the same as those under which the past record was made.

+.5 except in herds where the data extended over a time long enough to permit trends in management or in the genetic level of the herd to become important.

In unselected populations each cow is as likely to have had better-than-average environment as she is to have had worse-than-average environment. Therefore, in unselected populations the discrepancies between record and real ability are random. The plus discrepancies tend to cancel the minus ones and the average record of that population tends to equal (with some sampling error, of course, which may be large where the number of cows is small) the average ability of those cows under the average environmental conditions prevailing in that population.

But when a group of cows are selected because their past records were high, one tends to get not only cows which were above average in real ability but also cows which during those past lactations were exposed to better-than-average individual environment. If such selected cows are then kept for another lactation, whatever superiority in their former records was due to their superiority in real ability will tend to appear again but the cows will be exposed to a fresh sample of intra-herd variations in environment. So far as these environmental variations are temporary and random from lactation to lactation for the same cow, they are as likely to be minus as plus in the next lactation. Therefore, the average of the future records of a selected group of cows tends (with sampling errors, of course) to equal the average of their real abilities, but to be lower than the average of the records on which they were selected.

Similarly, when a group of cows is selected because their past records were low, the selection is for a result which may have been caused either by poor ability of the cow or by her having been subject to worse-than-average environment or by both. The average real ability (the average future records) of such a group will not be as low as the average of the records on which they were put in this low group.

Thus in the example cited from Edwards, the "high" cows and the "low" cows differed by 2,856 in the average of the records on which they were divided. But if in his data the repeatability was +.5 (the fraction to be used here would be higher than the usual repeatability of single records, because he used averages where a cow had more than one lactation), then the most probable difference between the real abilities of his two groups of cows would have been 1,428 and the expected difference between the two groups of daughters would have been 714, which is not vastly different from the actual 592. An r of .455, which is well in line with usual values, would have made the expected difference exactly what the actual difference was.

If the repeatability of single records in these published studies was approximately of the usual size, +.3 to +.4, most of the bias between the

sire indexes calculated on the two groups disappears when the proper correction is made for the effects which selection had in making records and real abilities of dams differ systematically; *i.e.,* for the regression of future records on selected records. However, since the actual repeatability of single records was not measured in the studies cited, it is not clear whether imperfect repeatability is the whole explanation for the daughters differing so much less than half as much as their selected dams did. The investigations reported in the present paper were undertaken to see whether still other circumstances need to be considered when using sire indexes for bulls mated to selected groups of dams.

There were two separate investigations nearly alike in procedure. The first was made by Jay L. Lush and Floyd Arnold and bits of the findings were published briefly in an abstract (7) and a press article (6). The other and more extensive study was made by H. W. Norton, III.[2]

ANALYSIS OF DATA

The First Study

These data were the 676 daughter-dam comparisons used in proving 103 sires in Iowa Dairy Herd Improvement Associations prior to January 1, 1937. All records were age-corrected, using the Bureau of Dairy Industry factors. Where the bull's mate had only one record the data for her and her daughter were discarded. The mates of each bull were then divided into a high half and a low half, solely on the basis of the *first* record of each cow. All the *later* records of each cow were then averaged into a single figure which was used in all subsequent computations of "later records." Thus each mate had equal weight in the average of later records, whether she had only one or several records after her first. If a bull had an even number of mates with two or more records each, all were used. If he had an odd number of such mates, the one whose first record was median in size was discarded and of course her daughter was discarded with her. This was done so that each sire would have exactly as many daughters and mates in the high group as he did in the low group. Therefore, differences in herd averages or in merit of the various sires could not affect the differences between high and low groups. If a mate had more than one daughter she was used again as many times as she had daughters.

Table 1 shows how the data were arranged for computation. Oakgrove Foxy was mated to eight cows which each had two or more records, but two of these cows had two daughters each, thus making ten daughter-dam comparisons available. The five mates with high first records are placed on the

[2] We are indebted to those in charge of dairy herd improvement association work in Iowa and to the Holstein-Friesian Association of America for making available in convenient form the daughter-dam comparisons on which these investigations were based, and to the American Dairy Cattle Club for assistance in the second study.

TABLE 1

Sample of data on fat production showing division into "high" and "low" groups

Sire which was being proved	High mate's records			Daughter's records		Low mate's records			Daughter's records	
	First record (X)	Later records		No.	Average (Z)	First record (X)	Later records		No.	Average (Z)
		No.	Average (Y)				No.	Average (Y)		
Oakgrove Foxy	417	3	436	3	420	270	2	366	3	410
	412	1	328	2	461	322	4	401	2	370
	372	5	365	2	476	323	3	367	1	442
	372	5	365	1	300	281	1	343	1	421
	323	2	380	1	330	281	1	343	1	261
Victory Flash	453	2	483	2	430	447	2	357	1	394
	476	1	494	1	397	326	2	458	1	438
	546	1	426	1	382	423	1	333	1	368
....	....	...	....	...	....	....	...	....	...	....
....	....	...	....	...	....	....	...	....	...	....
Averages (338 items in each column)	440.4		407.8		393.4	338.3		364.2		379.3

Repeatability of differences in single records $= \dfrac{407.8 - 364.2}{440.4 - 338.3} = .43$

Heritability of differences in single records $= \dfrac{2(393.4 - 379.3)}{440.4 - 338.3} = .28$

Heritability of permanent differences between cows $= \dfrac{2(393.4 - 379.3)}{407.8 - 364.2} = .65$

left while the five with the low first records are on the right. The mate's first record (X), the average of all the mate's later records (Y), and the average records of her daughter (Z) are shown in the same line. The rest of the study concerns the average values of X, Y, and Z for the high and for the low groups. Figure 1 shows graphically for both studies the values obtained for X, Y, and Z and the regression of Y and Z toward the average of X.

Table 2 shows the results obtained when the milk yields for the same cows were treated similarly. Naturally the distribution of the cows was not quite identical in tables 1 and 2, since fat percentage varied enough that some cows whose first fat records placed them in the high half for fat had first milk records which placed them in the low half for milk and an equal number whose first fat records were in the low half had first milk records which were in the high half.

The ratio of the Y difference to the X difference, .43 for fat and .48 for milk, shows what fraction of the variance among first records of cows mated to the same bull was due to permanent differences between the cows which made those records. The rest of the variance was caused by temporary environmental forces and circumstances which were different in later lacta-

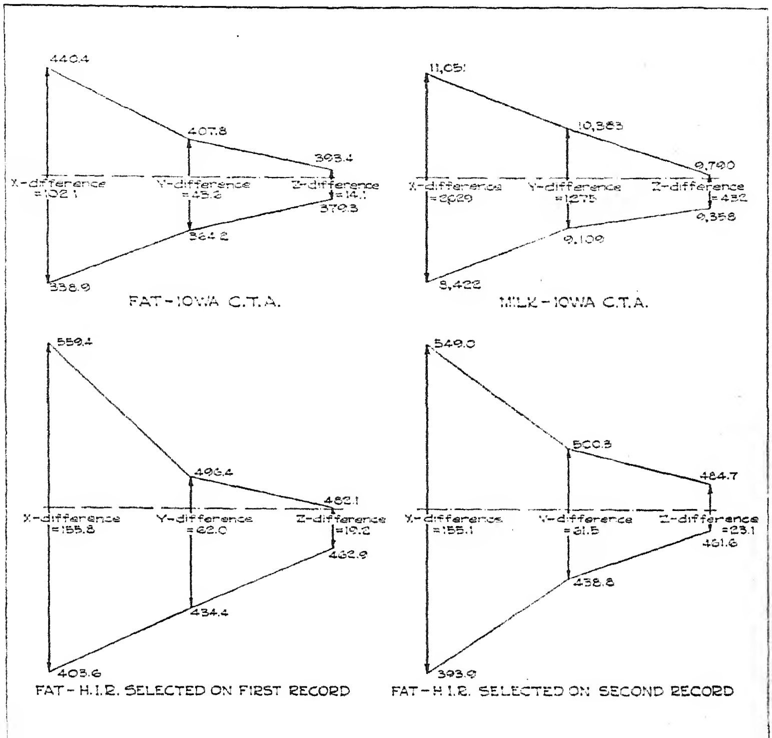

FIG. 1. Regression of later records and daughter's records from dam's selected record toward the herd average. Each horizontal line marks a level midway between the selected high records and the selected low records. The ratio of the Y-differences to the X-differences is the average repeatability of differences in the selected single records. Twice the Z-differences divided by the X-differences shows the average heritability of differences in the selected single records.

That the regression of the future fat records down from the selected high records is slightly greater than the corresponding regression up from the selected low records is interpreted as showing that some of the cows with low first records were culled before they could complete a second record, although the possibility of this being caused by a bias in the age-correction factors is not wholly excluded. That the daughters' fat records averaged below the first records of their dams and just about equal to or a little above the later records of their dams can hardly be blamed on any bias in the age correction factors, but it is in accord with the hypothesis that these dams are the survivors of some distinct selection practiced after their first records were made and that bulls bred to them were on the average just about equal to these cows in breeding value,—a bit better in the H.I.R. data.

tions. Because temporary circumstances had so much effect on the size of each record the later records (Y) regressed far toward the herd average. The real abilities of the cows differed only 43 (48 for milk) per cent as much as their records did.

TABLE 2

Average milk yields when data were divided according to size of the mate's first milk record

	Low group	High group	Difference
Mate's first record	8,422	11,051	2,629
Mate's later records	9,109	10,383	1,274
Daughter's records	9,358	9,790	432

$\frac{1274}{2629} = .48$ $\frac{2 \times 432}{2629} = .33$ $\frac{2 \times 432}{1274} = .68$

In terms of correlation, the ratio of the Y differences to the X differences approximates the intra-herd "repeatability" of single records, *i.e.*, the correlation between single records made by the same cow.[3] This repeatability coefficient describes the average condition within these populations, each of which consisted of cows mated to the same bull in an Iowa D.H.I.A. herd. Lower repeatability would be expected in populations where the cows were more uniform in their real ability or where individual environment varied more widely and irregularly from one lactation to another. Repeatability would be higher in populations within which the cows were more diverse in their real abilities or where intra-herd environmental conditions of all kinds were more rigidly equalized than here. Since each population in this study pertained to only one bull and included only those mates which had at least one tested daughter and two records of their own, and since there is little mixing of breeds in Iowa dairy herds, each such population is probably just a shade more uniform than a truly random sample taken from a whole breed but kept in the same herd would be. Cows leaving the herd before they finished two lactations could not appear here and such cows would include more than a fair share of the lowest producers. The group of cows mated to one bull would in many cases contain two or more which were half or three-quarter sisters by descent from preceding sires.

If the dam's own real ability were identical with her breeding value (which is the same thing as supposing that all differences between permanent abilities are hereditary and that the actual effect of each gene—that is, the phenotypic change produced by substituting it for its allel—is the same in every kind of genotype as it actually averages in that population), then the two groups of daughters by the same sire would tend to average half as far apart as their dams. Actually the daughters do not differ that

[3] Strictly speaking, it is an approximation to the regression instead of the correlation but where the standard deviations of first records and of second or later records are equal, as was approximately true in these data, the regression of future records on single records and the correlation between single records are identical. Estimating a regression by this high-half-versus-low-half method is not quite as efficient at getting out of the data all the information they contain as is the least squares method, but it is not consistently biased in either direction and may at times be simpler to compute and to explain.

much. Half the difference between the Y values is 21.8 pounds of fat and 637 pounds of milk, whereas the actual differences between the Z values are but 14.1 pounds and 432 pounds, or about two-thirds as large as they would be if all differences between the real abilities of the dams were additively genetic.

The other third of the permanent differences in ability can have been due to one or more of three things, quite diverse in principle but not separable from each other in the present data. First, some of the permanent differences between the dams may have been caused by environmental peculiarities which were permanent throughout the lifetime of each dam, or at least extended over more than one lactation. For example, if a cow's udder was damaged permanently by improper feeding, or by any other environmental cause before she made the first record, that would not alter her breeding value but would lower her producing ability in all lactations. Secondly, some of the dams might, on account of dominance, have permanent abilities higher or lower than correspond to their breeding values. If dominance exists, the daughters will on the average regress farther toward the mean of the herd than would be expected if dominance did not exist, regardless of whether it is the genes for high or for low production which are dominant or whether this is mixed, the gene favoring high production being dominant in some pairs and the gene for low production being dominant in others. Thirdly, some of the genes may in certain combinations produce more (or less) than their average effects; *i.e.*, some genes may have their effects made larger in some genotypes and smaller in others because of complementary, inhibitory, or other epistatic interactions with other genes. When the dam transmits a sample half of her genes to her daughter, many of these special combinations will of course be scattered. To the extent that the constituent genes can produce their effects only when all are together in those peculiar combinations, the daughters generally will not deviate as far from the breed average as would be expected from the permanent abilities of their dams.

Since these three diverse causes for a cow's real ability being higher or lower than her breeding value, together account for only about a third of the variance in the real abilities of a bull's mates (only about one-seventh of the variance in single records), and since it seems certain that some of this one-third is caused by permanent differences in environment, it appears that neither dominance nor epistasis (nicking) were highly important in causing the differences which existed among the cows mated to the same bull. Yet one cannot conclude that either of these two was totally absent unless he assumes that *all* discrepancies between permanent ability and breeding value were caused by environmental effects which differed from cow to cow among the mates of each bull but were the same from lactation to lactation for the same cow.

The Second Study

These data came from the first eight volumes of the Holstein-Friesian Herd Improvement Registry Year Book. All sires having at least six daughter-dam comparisons in which the dam had at least two records were used. There were 209 such sires[4] with a total of 3010 daughter-dam comparisons. Only the records of fat production were studied. All records were adjusted to maturity and to three-times-milking per day, using the conversion factors developed for that purpose in the Holstein-Friesian Advanced Registry Office.

The data were analyzed by the same procedure used in the first study. Then the process was repeated, this time dividing the dams into a high and a low half on the basis of their second record rather than their first. The first was then averaged with the third and later records, if any, to get an average of the cow's unselected records. This repetition of the analysis, but with the second record as the independent variable, was done merely to see whether the large regression from selected first records to future records was in any way peculiar to or dependent on the fact that the selected record was *the first* record.

The results are shown numerically in table 3 and graphically in figure 1. The regression of real ability on selected record was almost exactly the same (barely under .4) whether the dams were selected on their first record or on their second.[5]

The difference between the daughters is 3.9 pounds larger when the dams were sorted on their second records than when they were sorted on their first. This difference is probably too small and subject to too much sampling error to deserve much attention but it suggests that a cow's second record may be a shade better indicator of her breeding value than her first record is. Among first records 25 per cent of the variance seems to be hereditary in the narrow sense (additively genetic) while the corresponding figure for second records is 30 per cent. These two estimates are independent of each other, since the independent variables (dam's first record in the one case and dam's second record in the other case) do not include any of the same data. The two estimates agree reasonably well. They indicate (as might have been expected) that the moderately low repeatability and other findings from the first study were not peculiar results of using the first record instead of some later record for sorting the cows into "high" and "low" groups. Sorting on the second record gave almost the same

[4] If any of these were also proved in Iowa Dairy Herd Improvement Associations before 1937 they would have been included in the first study also.

[5] The close similarity of the two results is not surprising, since many of the cows had only two records. Had this been true of them all, the two regressions must have been equal unless the standard deviation of the first records was different from that of the second records. That is, the two figures are by no means independent estimates of the repeatability in these data.

TABLE 3

Average fat yields in data used for proving sires in Holstein-Friesian Herd Improvement Registry

	Low group	High group	Difference
When divided on mate's first record:			
Mate's first record	403.6	559.4	155.8
Mate's later records	434.4	496.4	62.0
Daughter's records	462.9	482.1	19.2
$\frac{62.0}{155.8} = .40$	$\frac{2 \times 19.2}{155.8} = .25$		$\frac{2 \times 19.2}{62.0} = .62$
When divided on mate's second record:			
Mate's second record	393.9	549.0	155.1
Mate's other records	438.8	500.3	61.5
Daughter's records	461.6	484.7	23.1
$\frac{61.5}{155.1} = .40$	$\frac{2 \times 23.1}{155.1} = .30$		$\frac{2 \times 23.1}{61.5} = .75$

results as sorting on the first. Presumably sorting on the third or later records would do the same except as the increasingly stringent selection, entailed by confining the study to cows with three or more records each, might reduce the amount of variance in the real abilities of the population which remained. Permanent but non-transmissible differences between cows are not a large fraction in either case—15 and 10 per cent, respectively, of the variance in single records, or 38 and 25 per cent of the variance in permanent abilities.

Comparisons with Other Studies

In none of the earlier studies in which mates were divided into a high and a low group was the repeatability of single records investigated. Therefore the present studies cannot be compared with them on that point. However, the present figures ranging from just under .40 to .48 do agree well with most studies of intra-herd repeatability in which the data had not extended over a long enough period of time for time trends to be important, or where the cows with early low records had not first been largely culled, as by restricting the study to cows which each had many records.

The earlier studies do permit computing the regression of daughters on dams. The difference between the daughters of the high and the low groups can be doubled and divided by the difference between the records on which the dams were separated into high and low groups. This yields a figure for heritability of differences among those first records, comparable to the 28 per cent for fat and 33 per cent for milk found by Lush and Arnold or to the 25 per cent and 30 per cent found by Norton for fat. Table 4 shows such a summary prepared from the earlier studies known to us. The heritability figures in table 4 are somewhat higher than those we found.

TABLE 4

Summary of evidence on heritability, earlier studies

Author	Characteristic	Difference between high and low groups		Heritability[a]	Notes
		Dams	Daughters		
Gifford	Fat (lbs)	278.7	32.2	.23	21 Holstein-Friesian bulls[b]
Gifford	Fat (lbs.)	240	61.6	.51	18 Guernsey bulls[c]
Copeland	Fat (lbs.)	244	52	.43	20 Jersey bulls[d]
Edwards	Milk (lbs.)	2856	592	.41	23 bulls[e]
Rice	Milk (lbs.)	6373	1815	(.57)	10 bulls, dairy breeds[f]
Rice	Test (%)	1.09	0.47	(.86)	10 bulls, dairy breeds[f]
''Brain Truster''	Milk (lbs.)	5025	945	.38	1 bull with 151 daughters

[a] Twice the intra-sire regression of daughters on dams.

[b] A.R. records. Each bull had at least 24 daughter-dam comparisons. The mates of each bull were divided into high, medium, and low thirds (approximately). The figures given here are averages computed from Gifford's table 12, giving equal weight to each sire.

[c] A.R. records. Each bull had at least 17 daughter-dam comparisons. Mates divided approximately in high, medium, and low thirds. The figures here are averages from Gifford's table 1, giving equal weight to each sire.

[d] R. of M. records. Each bull had at least 19 daughter-dam comparisons. Mates were divided approximately into high, medium, and low thirds. The figures quoted are from the summary of Copeland's table 3.

[e] Data from British milk recording societies in East Anglia and Lanarkshire and from agricultural college herds at Reading, St. Albans and St. Paul. Mates divided into high and low halves. The figures quoted are averaged from columns 4 and 5 of Edwards' table 3, giving each cow equal weight. As Edwards used average records where available (up to three lactations per cow), the heritability figure shown here pertains to differences between average records rather than single records. If the intra-herd repeatability of single records in Edwards' material was .4, the heritability of differences in single records would be somewhere between the .41 shown here and the .24 which would be approached if every mate had three records.

[f] Data are official records from several dairy breeds. Each bull had at least 17 daughter-dam comparisons. For each bull the five ''highest producing'' mates and the five ''lowest producing'' mates were selected. Division seems to have been primarily on total fat production and was for milk and test only in so far as they were dependent (statistically) on total fat production. This makes the records for the dams' milk and test come much nearer to representing the dams' real ability than if division into high and low groups had been primarily on the milk records and the test records respectively. The figures for heritability therefore are much too high to be fairly comparable with the others and come nearer to indicating the fraction of the differences in real ability (not records) which are due to additively hereditary differences between the cows.

We do not know whether this difference is statistically significant and needs an explanation. One possible reason for such a difference is that their data may have contained more inter-herd differences than ours. Most of the previous studies were confined to bulls which had an unusually large number of tested daughters. Doubtless that increased the proportion of cases where some of the dams and daughters were kept in one herd while others

were kept in another where the management differed.[6] This would have contributed an environmental portion to the daughter-dam correlation. Restricting the study to bulls which had very many daughters would also extend the time over which the daughter-dam comparisons had accumulated and would offer a little more opportunity for time trends in management to contribute to the observed correlation. Another conjecture is that our data, being D.H.I.A. or H.I.R. records, may have included a noticeable fraction of lactations made under circumstances abnormal enough that the owner would not have placed the cow on test if the matter had been left to his choice. That is, it is thinkable that other changeable circumstances do play a larger part—and genuinely hereditary differences consequently a smaller part—in such data as ours than in official test figures such as were investigated in most of the earlier studies. In any event the discrepancies between the figures for heritability in table 4 and those in tables 1, 2, and 3, are small.

DISCUSSION

Repeatability

By far the most important source of error in estimating the breeding value of cows from their records is the error which comes from conditions or circumstances which change from lactation to lactation for the same cow and make her production sometimes higher and sometimes lower than it will be if she is tested again under what are intended to be the same circumstances. The herdsman, or other person who knows the circumstances well, might be able to make some kind of allowance or correction for how the record has been affected by the unusual circumstances he knows (*e.g.*, difficult calving, a touch of milk fever or mastitis, freshening in fly time, two weeks of indigestion at what should have been the peak of her production, unusually good luck in avoiding a normal share of mishaps, etc.), but most such corrections will be so subjective that they can hardly be used by an impartial agency such as a breed association or D.H.I.A. supervisor. How far an observant herdsman's knowledge would actually go toward always explaining why a cow produced better in one lactation than she did in another is uncertain.

Lifetime averages are especially effective for correcting automatically the errors caused by unrecorded variations in environment. Variance due to circumstances which change at random from one lactation to another should be only half as large in averages of two unselected lactations, only one-third as large in averages of three unselected lactations, and so on, as

[6] Our own figures for repeatability and heritability are not absolutely free from that but must be nearly so since most bulls proven in Iowa D.H.I.A. before 1937 and most bulls proven in the first eight volumes of the Holstein-Friesian H.I.R. would have been proven in one herd only.

in single records. The process of averaging does not remove these errors entirely but, if they are random, it sharply reduces them.

Wherever objective correction factors can be developed for important variations in environment, further accuracy can be gained by correcting the original records for unusually good or bad known circumstances, but there are important practical reasons against correcting records too much, lest they get too far from reality.

Wherever simple and impersonal criteria of distinct abnormality can be devised, something might also be gained by omitting records made under circumstances so abnormal that no correction for them can be made (*e.g.*, abortion), but the circumstances justifying such omission would need to be few, definite, and unmistakable.

Heritability

Differences in single records seem to be somewhere between 20 and 50 per cent hereditary, the figures from our own studies being more nearly 25 to 30 per cent. It would of course be desirable to ascertain this figure more precisely and to know whether it is really different in different kinds of records.

Heritability was obtained by doubling the intra-sire regression of daughter on dam. The intra-sire basis was used in order to minimize the environmental contributions to daughter-dam likeness (since it puts most of the data on an intra-herd basis and restricts them to a period rather short for steady time trends to have had much influence) and to avoid analyzing the mating system. Questions of whether the mating system used was materially different from random mating among those selected to be parents were side-stepped by analyzing only the differences between cows mated to the same bull. The extent to which differences between groups of mates were hereditary was thus left unexplored. Genetic differences between groups of mates might be important in a population of partially inbred lines or in a population in which various herds were being selected toward widely divergent ideals.

Our estimates of heritability are a bit too high if there was any general tendency for the owner to give a daughter better environment than the average of the other daughters in his herd merely because he had given her dam better environment than the average of the other dams. Such a primary correlation between the environments of daughter and of dam would have contributed a non-genetic portion of the likeness between records of daughter and dam. We see no way of testing these data to learn whether such intra-herd environmental correlations between daughter and dam did exist but, in view of the feeding and management practices generally followed, we think that such environmental correlations must have

been unimportantly small, save only in the few cases where a sire was proved on daughters from more than one herd.

The definition of "hereditary" used here has included a small part of the epistatic gene effects, in addition to the purely additive ones. Additive gene effects were wholly included (aside from sampling errors), since each daughter gets a half of her dam's genes (and of course whatever average individual effects those genes have in the array of genotypes and environments present in this population), while doubling the regression tends to cancel the halving effects of Mendelism segregation. But only one-fourth of the two-gene epistatic interactions (*i.e.*, the differences between the effects which two non-allelic genes actually do have when together and the sum of their average effects, each considered singly in this population) which are present in the dam would be transmitted to her daughter, only one-eighth of the interactions peculiar to sets of three genes and not exhibited by any one or any two of those genes alone, only one-sixteenth of the four-gene interactions, etc. Hence the method of analysis used here has included in the 25 to 30 per cent of the variance which was "hereditary" not only the truly additive effects of genes but also about one-half of the effects which depended on the interactions of two genes, one-fourth of the effects of three-gene interactions, one-eighth of the four-gene interactions, etc. That such interactions may exist cannot be denied but in these data they can hardly have been very important, since one-half of the two-gene interactions, three-fourths of the three-gene interactions, seven-eighths of the four-gene interactions, etc., are included with the dominance deviations and the permanent environmental effects, which all together constituted only about 10 to 15 per cent of the variance.

Changing the Population Average by Culling Cows

Something about the rate at which the average production of a breed can be increased by culling low-producing females can be estimated from the fact that the intra-sire regression of daughter's record on dam's selected single record is something of the order of one-eighth to one-sixth in our data—a little higher in some of the other studies cited. The annual turnover in dairy herds is around 25 to 30 per cent of the average number of cows in the herd during the year. At least a third of those removals and possibly more than two-thirds are involuntary, being due to such things as old age, deaths, sterility, sales which would not occur if the cow were known to be well along toward calving, etc. If the voluntary selection which can actually be practiced is equivalent to discarding each year one-eighth of the cows which have the lowest records, the heifer calves sired the next year by the same bulls would average about two to four more pounds of fat per year when they come into production than the heifer calves from the preceding year would average. Seath in a study (10) of Iowa and Kansas D.H.I.A.

herds found that culling was not quite as intense as is assumed here. While a closer approximation is much to be desired, we think that these data and general considerations justify the opinion that the maximum amount of such culling of low producing cows as would be possible in herds generally, would not be enough to raise the average genetic productiveness of a whole breed at a rate as fast as three more pounds with each additional year. How rapidly the composition of a whole dairy population could be changed by the selection of bulls is another story and a much more complicated one.

Validity of Sire Indexes

Estimating the breeding value of a bull as equal to the average of his daughters plus the increase of these daughters over their dams rests on the genetic principle that the genotypes of the offspring tend to be midway between the genotypes (breeding values) of their parents and, in practice, on the inference that the average of the daughters' records equals the average of their genotypes and that the average of the dams' records equals the average of their genotypes under the general environmental conditions which are thought to have prevailed in that herd.[7]

[7] Because the interrelations between test (f), total milk (M), and total fat (F), are multiplicative but the segregation and recombination processes of heredity are additive, sire indexes are not quite identical when computed directly for F and when computed indirectly by multiplying together the indexes obtained separately for f and for M. The variables M, f, and F constitute a closed system in that when the values of any two of them are specified the values of the third is automatically fixed, but this does not of itself show which (if any) of them can be considered as primary or causal to the other or others. Several investigators have maintained that M and f are inherited independently and that F results from their interactions. This would make the indirect computation of indexes for F more nearly correct. Other investigators, notably Gaines, have maintained that the faint negative correlation (not far from $-.2$) generally observed between M and f is large enough to require explanation and is just about the size which would be expected if total energy yield (which is so closely correlated with F as to be almost synonymous, at least on an intra-breed basis) were primary. This would make the direct computation of indexes for F more nearly correct.

Whatever the physiological truth about that may be, the consequence of using whichever is the less accurate method of computing the sire index will be to throw into the epistatic portion some of the variance which would have been truly additive if the more accurate method had been used. The indirectly computed index for F exceeds the directly computed one by $2xy$ where x is the daughters' average test minus their dams' average test and y is the daughters' average milk yield minus their dams' average milk yield. Thus the indirectly computed index is larger than the directly computed one when x and y are both positive or both negative, but falls below it when x and y are of opposite sign. The actual size of $2xy$ in D.H.I.A. data was studied in a sample consisting of the first sire on each page in the list of proved sires published in August, 1940, as Miscellaneous Publication 393 of the U.S.D.A. For this group of 205 sires the average value of $2xy$ was -1.6 pounds and its standard deviation was 5.3 pounds. The two most extreme values found were $+18$ and -27 but only nine per cent differed from zero by as much as ten pounds and three-fourths of them were less than five pounds away from zero.

In the practical use of the index two sources of error are encountered. The first is the sampling nature of inheritance whereby the genotype of any one offspring may be better or poorer than the average genotype of its parents. Since the mechanism of inheritance makes such sampling errors always random, they are as likely to be plus as minus in each case and this source of error can be made unimportantly small (especially for characteristics affected by many genes) simply by increasing the number of offspring and seeing to it that they are an unselected sample.

The second difficulty is that the genotypes of the offspring and of the dams are unknown and can be estimated only from their records (phenotypes). So far as the differences between the individual's genotype and the individual's record are random, errors from this source also are as likely to be plus as minus in each case and can be made unimportantly small by increasing the number of dams and offspring. But in actual practice many of these discrepancies between genotype and record are not random but are more or less consistently biased in one direction. When these biases are large, only a little accuracy is gained by increasing the number of daughter-dam comparisons beyond three or four. Examples of such biased errors are: (1) that the daughters or mates of one bull are kept under an environment more favorable and another bull's daughters or mates are kept under a less favorable environment than the man interpreting the data thinks; (2) that the daughters or the mates used to prove the bull are a selected group—in which case their records tend to be better than their real abilities; (3) that sometimes only the highest record of each cow is used to represent her—in which case the records are generally better than what the cows will really do in the future (a source of bias which is more extreme for the mates than for the daughters since the former will generally have more records from among which to select a high one); and (4) certain rare (and therefore generally unimportant) genetic situations, such as an extreme change from intense inbreeding to wide outbreeding or the reverse, in which cases the daughters may be expected to show more heterosis or more inbreeding depression than their dams.

If the daughters were unselected their average phenotype can be taken as equal to their average genotype under the general conditions of that herd. The possibilities for error in doing this are: (1) random plus or minus errors caused by individual fluctuations in the environment which

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While of course it would be intellectually satisfying and scientifically interesting to know more certainly the relative accuracy of the two methods, yet for the practical purposes of choosing or rejecting one sire as compared with another the differences between the two methods are unimportantly small compared to sampling errors and to the ever-present possibility of incorrectly appraising differences in the general environment which prevailed either for the daughters or for the mates of any of the bulls being compared. The difference between these two ways of computing an index for F is of interest chiefly because it has sometimes been interpreted as challenging the general validity of indexes.

affected the various daughters and which may have made the actual records of some higher and of others lower than would generally be typical of cows with such genotypes, and (2) the general environmental conditions of that herd may have been better or poorer than is realized or than in the herd in which was proved another bull being compared with this one. Errors from the first source can be made unimportantly small merely by increasing the number of daughters. There is no such automatic way to guard against errors from the second source. One can only estimate this from records and observations of the feeding and management prevailing in the herds concerned. Such estimates can hardly be perfectly accurate but they are usually helpful, sometimes very much so.

It is now pretty well understood that for the progeny test to be unbiased the daughters should be an unselected sample—although that was not so generally conceded 20 or 30 years ago when arguments were frequently advanced that even a single high-producing daughter proved that a bull *did have the ability* to transmit that level and therefore he should be judged more by his best daughter than by the average of all—with substantially the same logic as is still sometimes offered in favor of using a cow's best record instead of the average of all her records! Yet it is never known that an actual group of daughters was absolutely unselected. The nearest approach to that is when every daughter born is tested. Even then some bulls might have transmitted more zygotic lethals, resulting in resorptions, abortions, or stillbirths, than other bulls. Often some of the daughters born alive die or are barren or are sold before reaching breeding age and no production record from them can ever be had. Pobably most of these omissions result from accidents or circumstances not related to the heifer's innate producing ability and hence do not tend to bias the average of the sire's progeny, but at least a few of these omissions result from constitutional weaknesses or culling for suspected low production, in part hereditary and having some bearing on the sire's breeding value. In short, natural selection *must* and intentional selection *may* impinge on the group of daughters to such an extent that the statement that the daughters must be unselected expresses an ideal which can be approached more or less closely and is very much worth striving for, but is rarely known to have been attained absolutely.

The mates have been exposed to all this selection and more, too. The more times a cow calves, the more chances she has to appear as a dam in the proving of a sire. For example, if about one-third of all calvings result in heifer calves which are raised and tested in the proving of some sire (probably a rather liberal estimate in view of stillbirths, death losses and barrenness among the one-half—approximately—of the calves which are heifers), only one-third of the cows which are culled before their second calving will appear as dams in daughter-dam comparisons. Of those cows

which calve twice, one-ninth would appear twice and four-ninths once as dams in daughter-dam comparisons, while four-ninths would not appear at all. Of cows which calve three times, one twenty-seventh would appear three times, six twenty-sevenths would appear twice, twelve twenty-sevenths would appear once, and only eight twenty-sevenths would not appear at all in sire proof. Thus the dams in the usual sire proof contain a disproportionately large share of cows which for many lactations escaped death or culling, and a correspondingly small share of those which left the herd early.

If the dams were selected solely on their past records in that herd, then the best estimate of each dam's genotype (G) is:

$$G = A + \frac{nh(D - A)}{1 - r + nr}$$

in which:

A = the average of herd in which the dam was tested or, more precisely, the average of the whole group out of which the dams were selected.
n = the number of lactation records for that cow.
D = the average of those n records.
h = the heritability of intra-herd differences on the basis of single lactations (*e.g.*, .28 for fat in these Iowa D.H.I.A. data).
r = the intra-herd repeatability of single lactations (*e.g.*, .43 for fat in these Iowa D.H.I.A. data).

For strictest accuracy the average of the above values for G should be used rather than the dams' actual average in computing the sire index, the formula for which then becomes:

$$\text{Index} = \text{Twice the daughter average} - A - \text{Average}\ \frac{nh(D - A)}{1 - r + nr}$$

Now if the dams were unselected the last term tends toward zero, the plus items and the minus items in that average being about equally numerous and tending to cancel each other and it doesn't matter whether D or A is used, as they are nearly equal. But if the dams were themselves a selected group D will exceed A more often than not and the last term will tend not toward zero but toward some figure determined primarily by the size of D – A and of h but also affected by n and by r. The sum of the last two terms lies between A and $\overline{D}$, being $\frac{nh}{1 - r + nr}$ of the way from A toward $\overline{D}$ when n is the same for all dams. Where lifetime averages for the dams are used, n will generally average between 2 and 4. The values obtained for h and r in the present study give values for $\frac{nh}{1 - r + nr}$ ranging from .36 to .45 when n is 2 and from .49 to .55 when n is 4. It therefore appears that in general the correct amount to be subtracted from twice the daughter average is not far from half way between A and $\overline{D}$ (perhaps a bit nearer the

former), if the values we found for h are typical. Lower values for h would move the correct figure nearer to A, while higher values would make it nearer to $\overline{D}$.

Whether it would be worth while to correct each cow's records to obtain her most probable breeding value (G) to use in place of D in the sire index would depend upon how much more accurate the index is thereby made and upon the labor (mostly clerical, of course) and other costs of making the correction. Where $\overline{D} - A$ is small (*i.e.*, where the dams were not in fact a highly selected group) there has been, of course, little error introduced by selection of the dams and little is to be gained by any correction for it. Where $\overline{D} - A$ is large the use of $\overline{D}$ in the index, without making any allowance for the regression of dams' probable breeding values toward the herd average, makes the sire's index lower than if he had been tested on unselected dams.[8] This will tend to make a sire's index lower than is fairly comparable with a cow's record but, for comparing one sire with another the existence of $\overline{D} - A$ introduces error only to the extent that $\overline{D} - A$ varies from sire to sire. How much variation is there in the intensity with which the mates of different bulls actually were selected? Seath's study (10) of 147 Iowa and 37 Kansas D.H.I.A. herds showed that those cows which remained in the herds at least one more complete year had averaged in the preceding year 16 and 14 pounds, respectively, more fat than the entire herd. (This would be $\overline{D} - A$ of the preceding formulae, so far as any one year's selection was concerned.) There were statistically significant deviations in culling intensity from herd to herd and yet these deviations were not extreme. For example among the 37 Kansas herds this figure varied only from zero to 33 pounds. We do not think such variations will often be extreme. In actual practice no one intentionally selects for low production. Variations in the intensity with which the mates of a bull have been selected occur only because men vary in the importance which they attach to production records, or in their biological and financial freedom to select. Only on rare occasions, as when someone with sufficient wealth assembles a foundation herd by picking a few high record cows from each of many herds, or at the opposite extreme when a man has permitted buyers to top out his herd and starts with the low record remainder to build his herd again, would variations in intensity of selection of mates be extreme.

Where it is suspected that one sire has been used on mates selected with unusual intensity, that can be verified by comparing the records of those mates with the average of their herd in the same years. That will entail more comparison of each cow's record with the herd average than is yet

[8] The increase of daughters over dams is even more severely biased by this circumstance. This seems to warrant some optimism in interpreting such findings as that nearly thirty thousand daughter-dam comparisons in the germ plasm survey reported in the 1936 Yearbook of the Department of Agriculture showed an average decrease of one pound of fat from the dams' records to the daughters' records.

customary, but perhaps no more than should be done. If such a comparison shows that correction for differences in intensity of selection is needed, the mates' records in each case can be brought nearer to their herd average by the formula for estimating G from D. There would be some complications in determining *exactly* how to compute the age-corrected herd average (A) for the comparable years but these will not prevent an approximation accurate enough for the present purpose. Perhaps we will eventually come to consider all records more on that basis, as has been urged to a limited extent in Scotland (byre average) or in Germany (Stalldurchschnitt). In small herds A will be erratic because of sampling errors. The values of h and r should be known with greater precision and, if they really vary much, their values in different kinds of populations need to be better known. In some cases selection may have been primarily for things other than the dam's records and, if those things were correlated with production, this has the effect of making the fraction $\frac{nh}{1-r+nr}$ nearer to unity (which would completely justify the use of $\overline{D}$ and the omission of A from the index) than it is when selection is entirely on the basis of records.[9]

Because increases in n make the fraction $\frac{nh}{1-r+nr}$ nearer to unity and diminish the size of $D-A$ which can be attained, the use of lifetime averages tends automatically to diminish (although it does not entirely eliminate) the bias which selection of dams introduces into the index computed as if $\overline{D}$ were identical with G.

Most of the discrepancy which selection of dams can introduce into the sire index disappears if the dams' records are properly corrected for incomplete *repeatability* (*i.e.*, even without going so far as to correct for incomplete heritability of permanent differences). For example in the present Holstein H.I.R. data, if a composite index for the high dams and one for the low dams is computed, using for each dam her first record (the one on which she was classified as high or low), we get with the low record dams an index of 522.2 and with the high record dams an index of 404.8, the difference being 117.4 which seems alarmingly large, since the bulls are the same. But if we discard the selected record and use only the future records of the very same cows (thus correcting completely for imperfect repeatability) the index with the low dams is 491.4 and with the high dams is 467.8, the difference being only 23.6 pounds which is a bias, to be sure, but is small compared to sampling errors and other sources of error in actual

[9] It should not be inferred that this hints at a short cut to more rapid improvement in production by selecting for something other than records of actual production. What is gained by increasing the size of $\frac{nh}{1-r+nr}$ will be more than lost by the smaller size of $\overline{D}-A$ which can be achieved if the dams are selected for something correlated with productivity rather than directly for their own records of production.

practice. Moreover, even this small difference was produced only by differences in selection of mates more intense (high half versus low half) than would often if ever be met in practice. That this remaining difference (23.6) does not approach zero is due to the fact that some of the differences between the records on which the selection was based were permanent for the cows concerned but were not transmissible to their daughters—*i.e.*, were due to permanent effects of environment, to dominance or to epistatic gene interactions. When these cows are sorted on the second record, the difference between the indexes on the "high" and the "low" groups is 108.9 when the mates are represented by the record on which they were sorted, but only 15.3 when the same cows are represented by all their other records, thus fully discounting the differences between record and real ability. In the Iowa D.H.I.A. data the differences in the fat index were 73.9 when the mates were represented by their selected records but only 15.4 when the same cows were represented by their other records. The difference in the milk index was 1765 pounds with the selected records but only 410 pounds when the very same cows were represented by their other records. These small remaining differences will disappear, too, if heritability (h) is known well enough that correction for it can be made.

It is pertinent to inquire whether there would be less error if A were used in place of $\overline{D}$ in the ordinary form of the index. This would be a little like the Swiss practice of proving a sire by comparing his daughters with the average of the association in which they were tested rather than with their own dams. This is done there to avoid penalizing a bull whose daughters are grazed at the higher altitudes and to avoid giving an unearned premium to the bull whose daughters are kept mostly on the richer pastures in the lower valleys. There will be less error in using A alone in place of $\overline{D}$ alone whenever the average value of $\frac{nh}{1-r+nr}$ is less than one-half. With n ranging from 2 to 4 and with the magnitudes of h and r which are usually encountered this fraction will generally be not far from one-half, probably a bit lower more often than it is higher. In general the larger n and the more h exceeds half of r, the more the advantage swings from using A toward using $\overline{D}$ if either must be used alone. Incidentally one can partially justify extending the index to include daughters out of untested dams, using the herd average (A) in place of the record of each such untested dam and D for each tested dam. This would remove one moderately important practical limitation on the use of indexes. Naturally it would be a coincidence if this happened to give $\overline{D}$ and A *exactly* the proper weights but the composite figure used would be somewhere between them, as the theoretically correct figure would be. Also of course in practice it would be necessary to be sure that the untested dams were in fact untested and that the records were not merely omitted because that would thereby give the bull a higher

index! Perhaps one wouldn't often know A where the dams were untested?

The most effective procedure for giving a bull a falsely high index through selection of his mates would be as follows: Assemble a group of cows which have only one record and that record an unusually low one in the herd in which it was made. Never test these cows again, or at least never use their subsequent records. Breed the bull to them and test his daughters when (some three or four years after the plan is started) they begin to freshen. Merely to state these requirements is enough to show that it would never be profitable for an unscupulous man deliberately to undertake this with the hope of making a profit on the extra price he would then get for the high index of his "proved" sire (if still alive) some four or five years after the plan was begun! Especially would this not appear attractive since the bull's daughters would probably not themselves be especially profitable since they would be out of dams poorer than average, even though not as poor as their records. Moreover the requirements (becoming more widely adopted) that lifetime averages must be used in proving sires, and that all cows in the herd must be tested, go far toward minimizing even this possibility.

The Sire Index Compared with the Daughter Average or the Daughter-Dam Difference

Space prevents a detailed comparison of the various ways proposed for comparing proven sires with each other, but the source of error which is the main object of this study,—more intense selection of the mates of some sires than of others,—biases the daughter-dam difference most, among the commonly used measures of a sire's worth. Selection of the dams makes the increase of daughter over dams too low by the quantity $(\overline{D}-A)\left(1-\frac{h}{2}\right)$ where the dams have only one record each and no correction for imperfect repeatability or heritability is made. The daughter average is biased in the opposite direction by the quantity $(\overline{D}-A)\frac{h}{2}$ which favors the bull mated to the most highly selected cows. Since the index is simply the sum of the daughter average and the increase of daughters over dams, these two opposite biases partly cancel each other in the index, leaving the net bias equal to $(\overline{D}-A)(1-h)$. For values of h lower than two-thirds this bias will be larger, when expressed in pounds, for the index than for the daughter average. But the standard deviation of indexes will be larger than the standard deviation of daughter averages,[10] ranging from only a

[10] The ratio of the standard deviation of the index to the standard deviation of the daughter average in populations in which each daughter and each dam are represented by one unselected record and the standard deviation of single records of daughters is equal to the standard deviation of single records of dams is:

little larger, in populations where the herd differences are extreme and the number of daughter-dam pairs is very large, to about twice as large in populations where averages differ little from herd to herd. When the bias from differences in the selection of dams is expressed relative to the standard deviation of whatever measure is used for the sire (as it should be for comparing the practical importance of a source of error), this bias would be equally serious in the daughter average and in the index when h is somewhere around .4 to .6. When the dams have more than one record each, the h of these computations would be replaced by $\frac{nh}{1-r+nr}$ which is larger. Therefore the general situation on this point is that the daughter average is usually a little less affected by selection of dams than the index is, especially when h and n are small, but this may be reversed when h is large relative to r, and when the dams have several records each.

The daughter average is most vulnerable and the daughter-dam difference is least vulnerable to errors from wrongly appraising differences in management and general environment from herd to herd, with the index again being intermediate but somewhat nearer to the daughter average.

Adjusting a Cow's Record for Imperfect Repeatability or Heritability

Corrections for imperfect repeatability are not usually made in practice, except when comparing individual cows which do not have the same number of records. For most other purposes one will be using an average of the records of several cows. Correcting for imperfect repeatability would lower some of these toward the herd average but would raise others. If the group of cows was unselected, these plus and minus corrections would tend to

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$$\sqrt{4+\frac{1+(n-1)(v-4u)-4t}{1+(n-1)w}}$$

in which n = number of daughter-dam pairs.
w = correlation between paternal sisters.
u = average correlation between daughter and a mate of her sire, other than her own dam.
v = correlation between cows mated to the same sire.
t = correlation between daughter and her own dam.

All these correlations are computed as if the whole population in which these sires were proven was a single unit (*i.e.,* they are not intra-herd correlations). u and v will be approximately the same size and will be roughly equal to the average correlation between herd mates. They are measures of the amount of herd heterogeneity in the population and in most dairy populations thus far studied are something of the order of +.2 to +.4. w will be larger than v by about $\frac{h}{4}$. t will be larger than v by about $\frac{h}{2}$. Where each cow is represented by several records all four of these correlations would be larger and this ratio would be noticeably smaller when n is very small but would not be changed much when n is large.

cancel each other and, therefore, little would be gained by making them. But where the cows were selected partly because they already had records higher than others in the population from which they were taken, more downward than upward corrections for imperfect repeatability should be made and therefore these will not tend wholly to cancel each other. The formula for making the repeatability correction is that a cow's most probable future ability is $\frac{nr}{1-r+nr}$ times as far from the average of the population as the average of her n completed records is, with r being the repeatability coefficient or correlation between different single records of the same cow in that population. Where $r=.4$, which is close to the value generally found for intra-herd repeatability, this fraction reduces to $\frac{2n}{2n+3}$, which is an approximation close enough and simple enough for general use.

If it is desired to estimate a cow's breeding value instead of her own ability (that is, to discount not only the temporary environmental differences in records but also differences which are permanent in that cow but not transmissible), the r in the numerator must be replaced by the heritability fraction (h) which, of course, will be somewhat less. Thus in the Iowa D.H.I.A. data the fraction for estimating the real future ability of the cow to produce fat becomes that her real ability probably is $\frac{.43n}{.57+.43n}$ as far from the herd average as the average of her past records is, while her breeding value should be estimated at $\frac{.28n}{.57+.43n}$ as far above or below the average of her contemporaries as her actual records average.

Age Corrections

Incidentally, these data have some bearing on the approximate correctness of the age conversion factors used. In the Iowa D.H.I.A. data the dam's first production record (not always her actual first lactation) averaged 389.4 while the later records averaged 386.0. This slight decrease could be interpreted as an average bias in the age correction factors used but it could also be interpreted (and more logically it seems to us) as the result of selection and subsequent regression toward the herd average. Cows with only one lactation could not appear in these data. If some cows were culled because their first lactation was low and before they could complete a second lactation, that would have made the average of first lactations here a bit higher than the abilities of the cows which made them. Cows culled because of unusually low first records could not be there to show the expected regression upward, while cows with unusually high first records would be kept and would show the corresponding regression downward. If the age corrections were completely unbiased and repeatability of single

records were .4, this decline of 3.4 pounds is the amount which would be expected to result from selection equivalent to discarding before they could complete a second lactation the five per cent of the cows which had completed the very poorest first records. This is just a little less intense than the selection Seath found. The average of later records (386.0) is not biased by selection, since all cows with two or more records could be included, provided only that they had tested daughters. In the Iowa D.H.I.A. data the age-corrected first milk records average 9736 and the later ones 9746. If selection for milk records was practiced, some compensating circumstance conceals the results of it, except that the daughters average only 9574. That might be considered either as evidence of selection among the dams or as indicating that the sires did not average as high in breeding value for milk production as their mates.

In the Holstein-Friesian H.I.R. data the first records average 481.5 and the second records average 471.4. This difference is equal to the amount which would be expected if the age correction factors were correct but in these H.I.R. herds selection between completion of the first lactation and completion of the second lactation had been of an intensity equal to culling seven or eight per cent of the cows with the very lowest first records.

Ward and Campbell in an interesting recent paper (11) interpret their data as showing that age corrections should be made by a regression equation and not by multiplication with a percentage correction factor. However, their method really corrects *both* for *age* and for *incomplete repeatability,* in a single operation. The equation for predicting from her first record (X) what a cow will most probably produce (Y) under a fresh sample of environment when mature is simply:

$$Y = (1 - r)A + raX$$

where a is the percentage age-correction factor appropriate for the age when record X was made, A is the mature average of that population, and r is the repeatability of single records. This corresponds to the equation under Ward and Campbell's table III except that their r of .64 is the correlation between first record and the average of the four later ones. This .64 corresponds to a repeatability of not far from .51 between single records,—.4139 is approximately equal to $\frac{4r^2}{1+3r}$, a little more or a little less according to inequalities among the various r's and σ's. This .51 is for the population as a whole and therefore (to the extent that these New Zealand herd averages differed from each other either because of environment or genetically) is larger than the intra-herd r's which we have been discussing here. The findings of Ward and Campbell do not conflict with the idea that corrections for age alone should be made by multiplying the actual record by a factor appropriate to that age.

Variability of Records, Abilities and Breeding Values

A cow's record, being the result both of her own ability and of the impinging environment, is more variable than either of these constituents. Similarly variation in real ability is greater than variation in breeding value. Quantitatively this is shown in Figure 2, using for illustration the

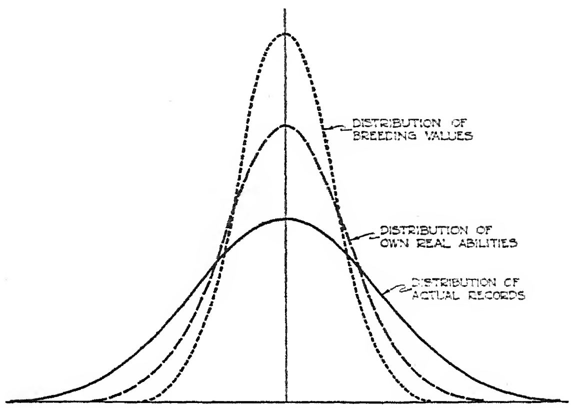

FIG. 2. Normal curves of equal area but with standard deviations in proportion to 100, 66, and 53. While it is not likely that the distributions of records, real abilities, and breeding values are exactly normal (distributions of biological data often being skewed a bit to the right or left for genetic or physiological reasons), yet they are nearly enough normal for this diagram to illustrate with fair accuracy the general principle that breeding values differ less than individual abilities and these in turn (at least for milk and fat production) differ far less than records of actual production. In general the cows with the very best records aren't as good as their records and the cows with the very worst records aren't as poor as their records.

Iowa D.H.I.A. data on fat where the repeatability was .43 and the heritability was .28. The standard deviation of real abilities is $\sqrt{.43} = 66$ per cent as large as the standard deviation of single records. The standard deviation of breeding values is $\sqrt{.28} = 53$ per cent as large as the standard deviation of single records. This leads to the estimate that the intra-herd variation in breeding ability (σ_G) was about 34 pounds of fat in Iowa D.H.I.A. cows and 49 to 53 pounds in H.I.R. data. About half of the difference between these two standard deviations would be expected to result from the H.I.R. data being corrected to a three-times-per-day milking basis. The rest of the difference may not be significant but it suggests that average H.I.R. management conditions are such as to expand the variation in records to a wider range than under C.T.A. conditions without, however, altering noticeably the proportion of that variation which is due to differences between the cows.

In any event these estimates give grounds for careful scrutiny of cases where a bull is supposed to have raised the production of his daughters over

their dams by more than 50 pounds of fat. Even in the H.I.R. conditions only one bull in 40 would be expected to do as well as that if his mates were average cows of the breed and not one in millions could achieve a real genetic increase of as much as 100 pounds. This is not to cast doubt on the accuracy of the records when such cases are reported, but to call attention to the great probability that the daughters were managed better than their dams, or were a selected sample, or that these dams had records far below their contemporary herd average, or that the number of daughter-dam pairs was not large enough to keep chance from playing a large rôle. The loopholes in sire "proof" are often large.

SUMMARY

The major source of error in estimating the breeding worth of cows or in interpreting the progeny test of bulls from production records is in environmental circumstances known and unknown which may make one record higher or lower than another, even for the same cow kept for another lactation under what are intended to be the same conditions.

Dominance, epistasis, and individual peculiarities of environment which affect permanently a cow's ability to produce fat are of minor importance in causing differences in the records used to prove sires. All three of these sources of variation combined are only about half as important as permanent differences which are simply transmissible from dam to daughter.

When the mates of a bull were divided into a high half and low half on the basis of one record and then the later records of the mates and the records of their daughters were compared, the sources of variance in single records were found to be as follows:

Percentage caused by	Iowa D.H.I.A. data (first lactation)		H.I.R. data Holstein-Friesian	
	Fat	Milk	Fat in first lactation	Fat in second lactation
Temporary variations in environmental conditions	57	52	60	60
Permanent but non-transmissible differences between cows	15	15	15	10
Hereditary differences between the cows	28	33	25	30

Differences in the intensity with which the mates of various sires were selected will bias sire proof, especially the daughter-dam difference and to a lesser extent the index and the daughter average, but this bias is rarely large enough to need much correction in actual practice. Nearly all of that bias can be removed, where necessary, by correcting the mates' records toward the average of the group from which they were selected, so as to

allow for the regression of real ability or transmitting ability on selected record.

The use of lifetime averages automatically corrects for much of the bias which in selected groups exists between the records on which they were selected and their real abilities.

Daughters whose dams were untested can be included in sire indexes, by using the herd average in place of the record of each such dam. Such procedure is more likely to improve than to lower the accuracy of the index, although there is some risk of the latter.

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DAIRY PRODUCTION MILESTONES

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On June 17, 1938, Dr. H. B. Ellenberger of the University of Vermont presented a paper in the grove next to the dairy building at the Ohio Experiment Station on "The Contribution of Production Research to the Advancement of the Dairy Industry." It seems rather ironical that three years later I should be asked by the General Program Committee to give a paper on the campus of the University of Vermont on "Dairy Production Milestones." Whether or not the Program Committee was justified in this move will be for you to decide later. Whatever your decision may be it remains fitting that we meet in a state where 60 per cent of the agricultural income is derived from milk, and on a campus from which considerable of our dairy knowledge has emanated.

To attempt to go back to the beginning of time and trace step by step the advances that have been made in the dairy production field would be not only too great a task but superfluous. For the purpose of this paper it is not necessary to know that the oldest records of dairying go back to 6000 B.C.; that the Old Testament has many references to cattle, milk, butter and cheese; that all cattle belong to the genus Bos (from which the affectionate term "Bossie" may have originated); nor even that trench silos existed in the days of Caesar. These are matters for classroom discussion—things with which you who teach are more familiar than I. We are more concerned with the dairy production picture as it exists today in this country, the events that have made it what it is, and what the future may hold.

In the beginning, and for some time later, the producer was the whole show in dairying, even after man learned how to make butter and cheese. Until about the middle of the nineteenth century practically all butter and cheese was made on the farm. The first cheese factory was established in 1851 and the first creamery came into existence five years later. From then on the shift was rapid and we find in the official records fifty years later that creameries were making a billion and a quarter pounds of butter a year and that cheese factories were turning out 300,000,000 pounds of cheese annually. What a change had come about!

This shift in labor distribution and responsibility was of great significance to the producer on the farm, for it allowed him more time for improving his cattle and his land. This, plus the coincidental developments occurring during the same period, brought about more changes in the course of

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one century than had occurred in the entire period of history preceding. Among the more important developments assisting in this change were inventions such as the cream separator, the mechanical milker, and refrigeration; discovery of the principles of genetics; the establishment and development of breed associations and other associations designed to spot superior animals and eliminate the boarders; development of feeding standards, nutrition, bacteriology, and chemistry, with the Babcock test the most significant chemical procedure affecting the conduct of the entire industry. We cannot forget either that improvements in agronomic practice contributed much to this rapidly changing dairy world.

The establishment of Federal and State-supported agricultural institutions and a Federal Bureau of Dairying has, of course, made it possible, through the agencies of research, teaching and extension, to discover the facts around which any sound enterprise must be built and to disseminate these, along with other bits of information, to all who would like to know, thus supplementing the original tool of progress—experience.

As a result of the milestones already mentioned and the many more yet to be indicated, where are we today? This can best be answered by comparing the farm dairy enterprise today with what it was 100 years ago, just before the great transition began. There wasn't much to the dairy business 100 years ago. Each farmstead had a few cows that were housed in the winter, when they were usually dry, milked when convenient, and fed whatever happened to be available—grass during the summer, and a little grain, straw, roots, and potatoes during the winter. Later, when corn was common, many cows wintered on corn fodder and a straw stack. The milk was consumed as such by the family or as butter and cheese, except in the case of large herds in the native pasture regions. A good cow in those days was expected to produce about 160 pounds of fat in a year. The highest record known was 12,000 pounds of milk a year. The average yearly production was about 2,000 pounds. Breeding and feeding were haphazard. There was no farm machinery. There were no tower silos. There was no alfalfa; there were no soybeans; there were no commercial fertilizers; there were no commercial feeds; there were no proven sires; there were no county agents, dairy extension men, dairy professors, or dairy specialists. The dairymen were on their own and cattle declined instead of improving until a few wise men like Wintrop Chennery, Phillip Dauncey, and W. G. Duncan imported cattle from abroad, kept them pure, and bred for desirable characteristics.

Today it is different and much more complicated. The dairyman of today must be a smart man to succeed, for he must be an expert in at least four major activities: farm management, production, marketing, and mechanics other than the baling wire type. Given some available labor, capital, land, buildings and fences, machinery, cows, and a few minor sources of cash income such as hogs, chickens, cash crops, or outside work,

the dairy farmer must utilize these resources in such a manner as to realize a profit.

The problems of production are concerned not only with milk but with calves and with crops. For all three of these disease control must be understood. Marketing machinery must be understood in order to make the best disposition of the product. Systems of buying milk must be considered, although we could hardly expect all the dairy farmers of the East North Central States, of which Ohio is one, to know that the "code" price of 100 pounds of milk delivered to evaporating plants is obtained by successively multiplying the monthly average wholesale price of 92 score butter at Chicago by 6, adding 2.4 times the monthly price of "Twins" on the Wisconsin Cheese Exchange, dividing by 7, adding 30 per cent, and multiplying by the butterfat content of the milk. The buying habits of the consumer must be known and sanitary requirements must be observed. For all this many skills are required, not the least of which is the ability to operate and repair farm machinery, without at the same time becoming a machine slave like the farmer, who, when the power failed while running a milking machine, called out the barn door, "Does anyone around here know how to milk a cow?"

Just how involved the dairy production field is can best be illustrated by the curriculum we expect a student majoring in dairy production to follow. At our meeting this year a report will be made on the subject matter that should be covered by a dairy production major. In the field of veterinary medicine alone it will be recommended that he attain quite comprehensive knowledge regarding anatomy, physiology, hygiene and preventive medicine, and pathology. Theoretically he should become fully versed in veterinary medicine as it applies to dairy animals. Multiply this one activity by the many others comprising the dairy production field and you begin to realize how complicated our course of training must be. The importance of physiology in understanding the chemistry and physics of milk secretion, with its intricate hormone relationships, is only too apparent in the literature of recent years, and advancement in both of these fields of endeavor constitutes a momentous milestone.

In spite of the complexities of the business, much progress has been made. In 1940 about twenty-four million cows in the United States averaged 4,575 pounds of milk and 181 pounds of fat. This is almost two and a half times the average of a hundred years ago. But that isn't the whole story, for in 1939 cows in dairy herd improvement associations averaged 7,977 pounds of milk and 323 pounds of fat. Also, we can now boast of a world's record production of 38,606.6 pounds of milk and 1,402 pounds of fat, made by Carnation Ormsby Butter King. This kind of boasting may not be of the right kind, however. We should be more concerned with the long-time production records of groups of cows rather than with unusual

records of scattered individuals. How far we can still go in this direction is indicated by the fact that on January 1, 1941, there were only 763,502 cows on test in 31,381 herds. This represents only slightly over 3 per cent of the cow population.

Further marked improvement activities in the development of the purebred business seems only natural. In addition to satisfying a "hankering" for owning purebred dairy cattle a real impetus seems to be present in the records of purebred herds in dairy herd improvement associations. Last year registered cows in D.H.I.A.'s produced 8,400 pounds of milk and 343 pounds of fat; grades produced 7,728 pounds of milk and 319 pounds of fat. These figures are based on 17,000 and 21,000 random samples of registered and grade records, respectively.

It has been estimated that there are about 175,000 registered sires in service. That's one registered bull for every 138 cows. At one time that would have seemed like an impractical and impossible ratio but with the coming of another milestone, artificial insemination, perhaps it is not. At the present time only one in ten of the purebred bulls born each year is registered. Some of those not registered would not be suitable herd sires but a great many of them are disposed of because there is no market for them. Steps to increase the demand for purebred bulls, together with proper use of artificial insemination, should result in more rapid advancement of the dairy breeds than has ever been possible in the past.

In the economic and agronomic phases of dairy production there have also been many milestones,—too numerous to discuss in detail by one not qualified to do so. The general trend in the economic field has been to reduce costs through more efficient production methods and to facilitate disposition of the product through cooperative effort. About 40 per cent of the milk leaving the farm is now handled by farmer cooperatives at some stage. Interspersed with more economical production procedures have, of course, been new equipment and machinery, new crops, new feeds, new fertilizers and fertilizer treatments, new methods of handling crops, of which ensiling has probably been of greatest importance, and systems of pasturing that have extended the grazing season through greater utilization of early spring and late fall pasture, and by the use of emergency crops to tide over dry periods.

Disease eradication is a field of activity in which the results are only too apparent. We are all familiar with the success of the tuberculosis eradication program. Area testing was completed on November 1, 1940. This meant that in this country bovine tuberculosis had been reduced to less than one-half of one per cent. That is the best record of any country in the world. The history of Bang's disease control is replete with startling discoveries and equally startling but usually inffective remedies. It was in 1913 that the Vermont Experiment Station qualified for the Blue Book

because it was then that the advocator (Rich) of methylene blue as a remedy for Bang's disease was busily at work at that institution. The test and slaughter program has of course been very effective. Within a period of six years of Federal and State cooperation 346 counties in 20 states were in modified accredited Bang's disease-free areas, and 220 additional counties were under test. That represents progress and every effort must be exerted to see that adequate Federal and State appropriations are made to continue this work. Along with this program there is now the additional procedure of calfhood vaccination which represents another forward step in disease control. The possibility of breeding Bang's disease resistant families is not to be ignored, in view of the progress that has been made in this direction with swine.

Mastitis is now our number 1 disease problem and offers a challenge to research that eventually will be met. We know pretty well how to prevent the spread of this disease from animal to animal, but how successfully to prevent or destroy the infection will be the aim of much talented effort.

The matter of housing was at first one of protection from bad weather. With our increased knowledge of sanitation and physiology many refinements have been made. The relationship between environmental conditions and physiological function is still vague, and perhaps when the facts are all known we will find that we have been pampering too much.

In discussing improvement in milk production it was intimated that proper selection and elimination of animals, as well as intelligent breeding, were responsible. Of course other factors were also at work, the chief one of which probably was improved feeding. Indeed, the phase of dairy production commonly included under feeds and feeding and nutrition affords a series of milestones that are rather well defined and, to one who has been working in this field, these naturally seem especially important. All will at least agree that no matter what inherited ability to produce a cow may have this will not be fully exercised unless she is properly fed.

The first attempt to make dairy cattle feeding something other than haphazard or inconsistent grew out of increased knowledge of agricultural chemistry and resulted in the formulation of feeding standards. Such names as Groven, Wolff, Lehman, Haecker, Kellner, Armsby, Savage, and Morrison are familiar to all students of dairy cattle feeding, for these are the ones who provided the basis for scientific feeding. Protein and energy are the only requirements to be met in a feeding standard and for a long time it was felt that nutritive ratio, an expression of the balance between digestible crude protein, digestible carbohydrates, and digestible fat, was the all-important thing. Balanced rations were the vogue. Now we know that a ration must be not only balanced but complete. Our present feeding standards are probably neither infallible nor final. Even now a new system is

being developed for evaluating feeds, using casein and glucose as reference materials.

Much work has been done on the protein requirement of dairy cattle with the trend being downward with respect to actual amounts needed. Whereas it was once common for grain mixtures to contain 24 per cent or more of total protein it is now common for grain mixtures to contain 12 to 16 per cent total protein and give as good results. This represents an enormous saving, since protein is the expensive ingredient of purchased feeds. The science of nutrition has shown what the end-products of protein digestion are and which of these amino acids are essential for some species. As yet, however, the amino acid requirement of cattle is not known and may be of little importance since the microorganisms of the rumen are capable of converting simple nitrogen compounds into complex proteins. Urea and ammonium salts may some day be important nitrogen sources for dairy cattle.

Digestibility and balance trials, feeding experiments, and net energy determinations have contributed much in rapid succession to our knowledge of dairy cattle nutrition, but no one of these was more important than the now famous Wisconsin experiment in which good results were obtained on a ration made entirely from the corn plant and disastrous results when the wheat plant only was used. The difference between these rations could not be detected by chemical means and suggested that the then current basis of formulating rations was inadequate. This experiment, the results of which were published in 1911, stimulated the use of purified diets with small animals, a procedure which resulted in the discovery of the first vitamin in 1913.

The discovery of vitamins certainly was another milestone in dairy production, although what at first seemed a very complex and critical problem has resolved itself, up to the present, into one of quality in roughages. We now know that dairy animals need the fat-soluble vitamins A and D in their feed, but the simple procedure of furnishing pasture and sunlight in the summer, sun-cured hay of high quality and some kind of silage in the winter, seems to satisfy the body needs for these factors, except possibly in the case of calves born in the fall and winter until such time as they are able to consume two or three pounds of hay.

Of the water-soluble vitamins, those comprising the vitamin B complex offer no problem since it has been demonstrated that thiamin, riboflavin, pantothenic acid, and pyridoxine are synthesized in the rumen. Vitamin C is probably synthesized somewhere within the body and cannot be said to offer a problem in dairy cattle feeding in spite of the beneficial effects on reproduction of vitamin C injections. Within the vitamin field itself demonstration of the remarkable rejuvenating effect of injecting vitamin C into bulls and cows suffering from some form of reproductive failure is one of

the most far-reaching contributions that has been made to economical production.

Vitamin E still affords a field for fruitful research, especially since alpha-tocopherol, the pure substance, is now readily available. Although some European work is quite persuasive as to beneficial effects on reproduction in cattle injected with vitamin E in the form of wheat germ oil, there has been no work on this continent that indicates the need for more vitamin E than is found in natural dairy feeds. This is not proof that additional vitamin E might not be beneficial, and in view of some favorable preliminary work at Iowa with ewes fed wheat germ oil by capsule we must withhold judgment and keep an open mind on this question.

Long before the vitamins were discovered the importance of certain constituents of ash was being demonstrated. Calcium and phosphorus were found to be the principal constituents of bones and their need was readily shown by restricting their intake. Later, their relationship to vitamin D became clear. What milestones they were when the first mineral balances with cattle were run and the actual requirements for calcium, phosphorus and other inorganic substances were determined in many successive experiments. The work with minerals, just as that with vitamins, has taught us the importance of good roughage and has prevented much of the exploitation of individual or "shotgun" mineral and vitamin preparations. At the same time the research has been broad enough to detect the conditions under which additional calcium, phosphorus, iodine, iron, copper and cobalt are needed.

For a long time the fat in feeds was looked upon only as a source of energy, yielding 2¼ times as many calories as the same amount of protein or carbohydrate. With the discovery of the fat-soluble vitamins and of the indispensability of certain fatty acids, the importance of fat assumed greater significance. This was climaxed by the demonstration that fat played some role in milk secretion. Today the amount of fat in dairy feeds is receiving the attention of research workers, farmers, and feed manufacturers.

We have seen many developments in feeds themselves. Processing of all kinds has been tried to improve the original value of feeds: cooking, steaming, enzymatizing, sprouting, chopping, grinding,—just to mention a few. As a rule it has been found that the cow herself is the best processor.

No discussion of the role of feeding in the development of dairy production would be complete without some mention of the commercial feed industry. Many dairy farms could not operate at a profit without having available when needed commercial feed mixtures to supply the nutrients and energy their own farms did not provide, and most dairy farms would not like the prospect of not having readily available a few individual feeds such as wheat bran and one or more of the oil meals. The manufacturers of commercial feeds have much to offer to the farmer and to those who are concerned with his education and improvement. It is gratifying to see the fine rela-

tionship that now exists between (reputable) commercial feed men and those in the educational field, including research. Further improvement of this relationship will prove mutually beneficial.

We now come to a consideration of the material—milk—for the production of which everything that has been said before has some significance. Oliver Wendell Holmes said once that "a pair of substantial mammary glands has the advantage over the two hemispheres of the most learned professor's brain, in the art of compounding a nutritive fluid for infants." That may have been true once but no one can now deny that our present knowledge makes it possible for use to provide the materials from which a better nutritive fluid for infants will be produced. Probably the first clue to this possibility came in 1905 when an investigator in Holland, after finding that the addition of milk to a diet of casein, albumin, rice flour, lard, and a mixture of all the then known essential inorganic salts, made the difference between life and death in mice, wrote "there is a still unknown substance in milk which, even in very small quantities, is of paramount importance to nourishment." Should the writer, Pekelharing by name, be alive today and realize that his unknown substance was really a combination of now recognized vitamins, he would feel much like the piscatorial creature his name simulates.

For a long time the value of milk as a food was taken for granted. Consequently, emphasis was placed on improving the sanitary aspects of milk production. Can't you just picture some of the old town meetings at which some patriarch would get up and say "We're going to have clean milk in this town if we have to take the bull by the horns." As a result of outbursts like this such great advances have been made in the sanitary aspects of milk production that the consumer in communities where milk control is exercised is practically assured of the safety of the milk he drinks.

As the science of nutrition was developed and new hitherto unknown factors that contribute to the value of foods were discovered, the value of milk as a food was reinvestigated. As a result of this newer knowledge of nutrition, many of the things formerly taken for granted have been shown to have sound scientific basis, and other favorable attributes not previously known have been brought to light. At the same time one or two weaknesses or deficiencies were encountered that made it possible better to understand the limitations of milk, as well as its virtues. Knowledge such as this regarding any food product results in more intelligent use of it.

It would be presumptuous for me to attempt to enumerate those factors in milk that make it "the most nearly perfect food," other than to remind you that the concentration of certain vitamins in milk can vary several hundred per cent by employing special conditions such as the feeding of irradiated yeast to increase vitamin D and the use of grass silage for retaining summer vitamin A potency and esthetic appeal.

One approach to an evaluation of the nutritive value of milk as it is produced might be justifiable. There are eleven nutritional factors needed by humans concerning which quite definite information is available. These are calories, protein, calcium, phosphorus, iron, vitamins A and D, ascorbic acid, thiamin, riboflavin, and nicotinic acid. If the required amount of each of these eleven nutrients is considered as 1, and the amount of each nutrient furnished by a quart of milk is given a fractional part of 1, it will be found that 5.5 of the 11.0 units have been supplied.

This does not mean that two quarts of milk will satisfy all the dietary requirements, because at that rate of consumption calories, iron, and certain vitamins would still be short, and there would at the same time be a great excess of other factors, particularly calcium and phosphorus. It does point out strikingly, however, that because of the variety of nutrients furnished, liberal use of milk makes easier the selection of foods that are needed to round out the diet and make it complete.

The comparison just made was based upon milk as it leaves the cow. By the time that milk has been handled, processed, and handled again, some of its original nutritive properties have been lost. More detailed studies of these losses are needed and methods for preventing them must be devised. Otherwise we may some day find ourselves in the same predicament as the millers who after years of milling out some of the most valuable portions of the wheat kernel are now all enthused, with government encouragement, about putting those things back into flour.

That naturally raises the question of mineralization and vitaminization of milk. In a paper given at the Illinois meeting of the American Dairy Science Association in 1933, after justifying the fortification of milk with vitamin D, I made the following statement:

> The general mineralization and vitaminization of foods, including milk, would further complicate an already complicated situation. Vitaminization and mineralization of foods probably cannot be justified except where natural foods fail to furnish these vital factors. This is especially true of milk. To add various vitamins and minerals to milk haphazardly would . . . jeopardize the unique and excellent position which this product now enjoys in the eyes of the general public and the medical profession. In spite of the intriguing mystery and glamor that surround some of the newer discoveries in nutrition we must not lose sight of the fact that plain, ordinary milk is the best single food we have and is thus considered by all. The fact that a sufficient intake of calcium cannot be obtained except by the inclusion in the diet of some form of milk or cheese places these dairy products on a pedestal by themselves.

I still believe that statement, knowing full well that good arguments to the contrary can be advanced.

Let us consider what possibilities there may be. I have here a series of vials, each of which contains a pure vitamin or the purest known form. With our knowledge of chemistry where it is, how simple it would be to add any or all of these to milk without impairing its flavor and possibly improv-

ing its appearance! Should promiscuous fortification of milk be employed the only purpose of feeding cows would be to produce pounds of milk regardless of its food value. Fortunately, however, the present trend of agriculture, plus knowledge gained by experience and research, leans towards systems of feeding that not only produce large quantities of milk but impart to that milk the highest nutritive value.

But why pay any attention to the food value of milk? Do you think for a moment that milk food value education is going to increase milk consumption in a hurry? Not at all, unless a carefully planned, high-class radio program on a national hook-up is used. Education at best is a slow process. If you really want to increase milk consumption reduce the price of milk to the consumer or increase the payrolls. The city of Akron, Ohio, is now at the top of the list with respect to per capita milk consumption. A year ago it wasn't. What happened? For one thing industry has picked up; for another, milk has been sold in gallon jugs for 7, 8, and 9 cents a quart. Take a look at evaporated milk. In 1921 canned milk consumed was 9 per cent of the liquid milk purchased; in 1939, canned milk constituted 13 per cent of the total purchases of milk, *i.e.*, fresh and canned combined. It is quite safe to speculate that even more evaporated milk would be consumed if the flavor were better. I am not advocating jug milk and evaporated milk; I am merely trying to illustrate what price will do.

This becomes more striking when we realize that in normal times nearly two-thirds of our families have incomes of less than $1,500 and that the average income of these families is only $826. Nearly 42 per cent of our families provide only 26 per cent of our food market. That is the explanation of the paradox of want in the midst of plenty. A study of the food purchases of families as related to income shows that the consumption of evaporated milk actually goes down while that of bottled milk goes up as income rises above $750 a year. To what other conclusion can one come than that incomes must be increased or the price of milk reduced if the milk consumption level is to reach the point that seems best for conservation of our greatest natural resource—our people?

If there is a keynote to this address, it is this: economical production and, by inference, economical handling all along the line. I am amused at some of the reports that come to my desk as, for example, the one that listed the estimated price of all milk delivered to a particular market as $2.00 per hundred and the cost of producing that milk, using the Michigan formula, $1.98 a hundred. And yet many of the producers in that milk shed were making money because they were better than average. To raise the average we must have better land, better feed, better cows, and better men. It is our responsibility to help others attain all these things, and that probably means that in the future we will need to work more with the below average man than in the past.

As we meet here this week may we do so in a spirit of thankfulness that our work can be aimed at individual improvement rather than at the welfare of the State or party, for if the world wants to preserve science as a powerful social force for good the research man must be permitted to work without intellectual restraint, *i.e.*, he must be permitted to enjoy the fundamental freedom of democracy.

THE THIRTY-SIXTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ
Secretary-Treasurer

The American Dairy Science Association assembled in the gymnasium on the campus of the University of Vermont in Burlington on Tuesday, June 24, 1941, at 9:30 A.M.

The Honorable Warren R. Austin, Senior U. S. Senator and Trustee of the University, was introduced and delivered the address of welcome.

President Harry W. Cave then gave the following response:

"Senator Austin, I can assure you that we all appreciate very much your kind words of welcome, and the many things which have already been done to make our visit to Vermont pleasant and our meetings a success. Some of us felt that we were coming a long way from home when we came here but the cordial welcome we have received has made us forget that. I only wish that every member of our Association might be here to enjoy your delightful state and the many courtesies being shown us.

"We have come to New England for our thirty-sixth annual meeting because it is a definite policy of the American Dairy Science Association to hold its meetings at various rather widely separated points. In 1939 the annual meeting was held in the far West, then last year in the middle West and now in the East. This plan should make it possible for even somewhat isolated members to attend at rather frequent intervals.

"To further increase the benefits of the Association to its membership, there have been formed three branches of the organization known as the Eastern, the Western and the Southern Divisions. These divisions each hold an annual meeting with a program of contributed papers and a discussion of problems peculiar to the area concerned.

"Last year a committee was appointed from the Southern Division to prepare a history of that organization. This committee, consisting of J. A. Gamble, L. A. Higgins, C. A. Hutton, and J. A. Arey with W. E. Wintermeyer as chairman, presented their report at the annual meeting of that Division held in Atlanta, Georgia, on February 5–7, 1941.

" 'The Southern Division of the American Dairy Science Association had its inception at an informal conference in the hotel room of J. A. Gamble of Maryland during the National Dairy Show which was held at St. Paul, Minnesota, in October, 1921. Those meeting with Professor Gamble to discuss the proposed organization were C. W. Holdaway and F. A. Buchanan of Virginia, L. P. LaMaster of South Carolina, and C. A. Hutton of Tennessee. This group decided to make an effort to establish a Southern

Division and that letters concerning the proposition should be prepared, calling a meeting to be held during the Southern Agricultural Workers Conference scheduled for Atlanta, Georgia, on Feb. 20, 21, 22, 1922. The following letter prepared by J. A. Gamble and C. W. Holdaway was mailed to each dairy worker in the South on February 1, 1922.

Blacksburg, Va.
February 1, 1922

Subject: Meeting of Southern Workers in Dairying at the Association of Southern Agricultural Workers, Atlanta, Ga., February 20, 21, 22, 1922.

Dear Sir:

Arrangements have been completed for the meeting of the workers in dairying in the South for the purpose of forming a Southern Division of the American Dairy Science Association.

Every worker in dairying within the scope of this Division, which should include Maryland and West Virginia on the north, and Kentucky, Tennessee, Arkansas and Texas, and all States south and east of these, should be at the meeting. Every worker is an important factor in the dairy development of our Division and should emphasize his importance by being at this meeting.

The time and place will be on the afternoon of the 21st of February at the Piedmont Hotel.

A few short addresses will be given to emphasize the importance of this organized movement.

If for any reason you cannot be there, mail a letter to me at the Piedmont Hotel enclosing, first, your application for membership in the American Dairy Science Association if you are not a member already, and secondly, your vote on affiliation with the Southern Division when it is formed.

Very truly yours,
(Signed) C. W. Holdaway,
Professor of Dairy Husbandry.

" 'On Feb. 3, 1922, J. A. Gamble, as chairman of the membership committee of the American Dairy Science Association, mailed a letter to the following dairy department heads:

H. E. Dvorachek, Arkansas	M. R. Tolstrup, S. Carolina
M. P. Jarnagin, Georgia	C. E. Wylie, Tennessee
J. J. Hooper, Kentucky	J. A. Clutter, Texas
J. M. Cadwallader, Louisiana	C. W. Holdaway, Virginia
L. A. Higgins, Mississippi	E. L. Anthony, West Virginia

R. H. Ruffner, N. Carolina

" 'In this letter Professor Gamble called attention to the Atlanta meeting and enclosed blanks of application for membership in the American Dairy Science Association. A form letter containing information about the Association and an invitation to eligible dairy workers to join was also enclosed.

" 'As announced in the letter sent on Feb. 21 to all workers, the organization meeting of the Southern Division was held on Feb. 21, 1922.

" 'The following are the minutes of that meeting:

The first meeting of the southern members of the American Dairy Science Association was held at 4 P.M., in Room 911, Piedmont Hotel, Atlanta, Ga., Feb. 21, 1922. The members present included:

C. W. Holdaway, Virginia	J. A. Gamble, Maryland
C. E. Wylie, Tennessee	Stanley Combs, N. Carolina
M. P. Jarnagin, Georgia	L. H. Marlatt, Georgia

In addition to the members of the Association, J. P. LaMaster, South Carolina; J. M. Scott, Florida; R. C. Curtis, North Carolina; Dr. E. S. Good, Kentucky; J. C. Grimes, Alabama; C. E. McWhorter, Georgia, and several other leaders in the dairy and animal husbandry work in the Southern States were present.

The meeting was called to order by C. W. Holdaway of Virginia and J. M. Scott of Gainesville, Fla., was named temporary chairman. C. W. Holdaway was nominated and elected president; C. E. Wylie of Knoxville, Tenn., vice-president; and J. A. Gamble, College Park, Md., secretary-treasurer.

The first business of the meeting was to have the proposal explained and to pass a resolution asking that the executive committee of the American Dairy Science Association grant permission for the formation of a Southern Division of that body. Letters in support of such a proposal were read from several animal and dairy husbandry workers who could not be present.

It was moved that a committee of one be instructed to draft suitable by-laws and present them at the next meeting for discussion and adoption. J. A. Gamble was designated to bring in these suggested by-laws. It was further moved that as soon as the approval for the formation of the Division was received, C. W. Holdaway, the presiding officer, appoint the necessary committees—the committee list of the American Dairy Science Association to be used as a guide in the matter.

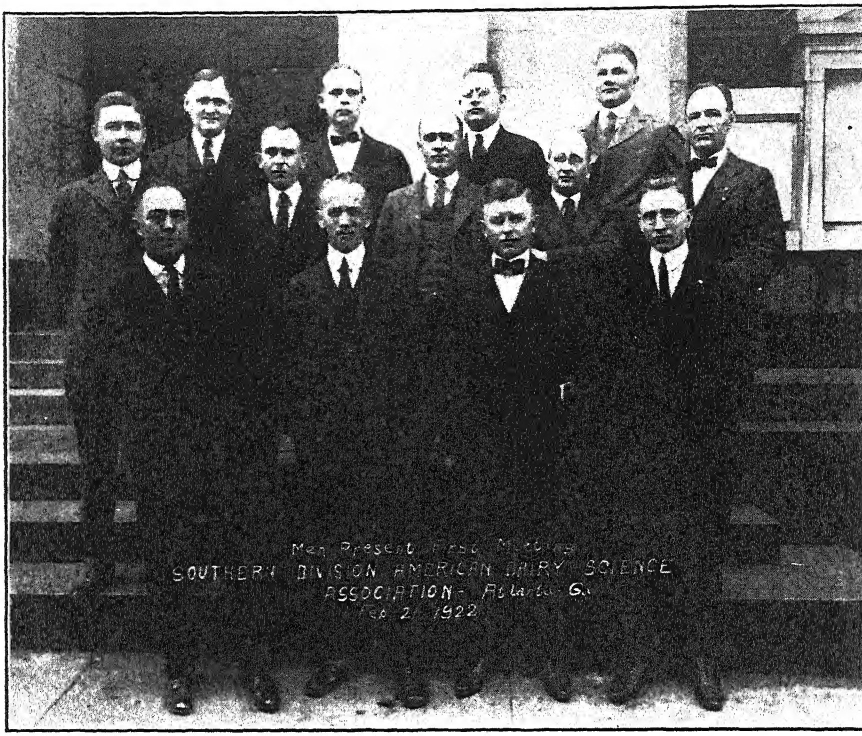
All present took part in the discussion relating to the opportunities for service of such a group in the improvement of dairy conditions in the Southern States. It was the general opinion that such an organization would not only result in the coordination of teaching, research, and extension work in the subject, but result in activities such as sectional dairy cattle and dairy products judging contests, to be held once a year at some central point.

The meeting of the group adjourned at 6 P.M.

(signed) J. A. GAMBLE,
Secretary-Treasurer.

" 'A second meeting was held the next morning, Feb. 22, 1922, at which the proposal to sponsor dairy cattle and dairy products judging contests was discussed. A committee was appointed to present the matter to the superintendent of the Southeastern Fair Association and report back.

" 'Arrangements were made for a picture of the newly organized group to be taken on the steps of the Carnegie library at 12:30 P.M. and copies of this picture have been preserved.



TOP ROW—E. L. Jordan, La.; S. Combs, N. C.; McWhorter, Central of Ga. R. R.; Rigdon, Central of Ga. R. R.

SECOND ROW—C. E. Wylie, U. of Tenn.; J. P. LaMaster, Clemson Coll.; J. F. Bazemore, Central of Ga. R. R.; E. S. Good, U. of Ky.; John M. Scott, Fla. Agr. Exp. Sta.

BOTTOM ROW—L. H. Marlatt, U. of Ga.; C. W. Holdaway, Va. Polytech. Inst.; J. A. Gamble, U. of Md.; L. J. Horlacher, U. of Ky.

" 'Under date of Feb. 24, 1922, J. A. Gamble, the newly elected Secretary-Treasurer, wrote President C. H. Eckles of the American Dairy Science Association requesting permission from the Executive Committee to organize a Southern Division of the Society. He enclosed twenty applications for membership.

" 'Favorable action on the part of the executive committee was reported by J. B. Fitch, secretary-treasurer of the Association, in a letter to J. A. Gamble dated March 16, 1922.

" 'The officers of the newly formed division arranged a program of papers and discussions for a regular meeting held Feb. 6 to 8, 1923. It is significant that the first subject under discussion was that of pasture grasses and pasture management for the South, presented by C. E. Piper, Forage Crops Division, U. S. Department of Agriculture. Attention given this

subject by early leaders of the Southern Division has helped to give it much needed impetus in the Southeastern states. Other papers presented at this and later meetings had to do with problems in teaching, feeding, breeding, etc., peculiar to the South. Each year a meeting is held during the annual convention of the Southern Agricultural Workers and the division has continued to grow in size and its activities.

"'The chairmen who have served the Southern Division since C. W. Holdaway in 1923, have been:

C. E. Wylie, Tenn.	1924	R. B. Becker, Fla.	1933
J. P. LaMaster, S. C.	1925	A. D. Burke, Ala.	1934
C. A. Hutton, Tenn.	1926	R. H. Lush, La.	1935
J. S. Moore, Miss.	1927	E. C. Elting, S. C.	1936
J. S. Moore, Miss.	1928	A. H. Kuhlman, Okla.	1937
A. C. Baer, Okla.	1929	C. N. Shepardson, Tex.	1938
R. H. Ruffner, N. C.	1930	T. B. Harrison, Tenn.	1939
L. A. Higgins, Miss.	1931	C. G. Cushman, S. C.	1940
Earl Weaver, Okla.	1932	R. E. Waters, Miss.	1941

"'The secretaries since J. A. Gamble, who served during the period 1923 to 1926, have been:

J. P. LaMaster	1927	A. H. Kuhlman	1935
A. C. Baer	1928	C. N. Shepardson	1936
L. A. Higgins	1929	T. B. Harrison	1937
J. S. Moore	1930	C. G. Cushman	1938
R. B. Becker	1931	R. E. Waters	1939
A. D. Burke	1932	C. D. Grinnells, N. C.	1940
R. H. Lush	1933	R. B. Becker	1941
E. C. Elting	1934		

"The object of our Association should be to serve dairy workers and the dairy industry as universally as possible. Our field of service has been expanded and our membership greatly strengthened through these branches of the parent organization, the Eastern, the Western, and the Southern Divisions.

"I would not close without expressing my sincere appreciation to the officers, the directors, the many committee members, and the individuals who have assisted in carrying on the work of the Association during the past year. Our organization is steadily growing and its work is constantly becoming more complex. The point has long ago been passed where this work could be done by a few officers. It has been a real pleasure to me to find the great willingness with which the many who were called upon, accepted the duties requested of them.

"We are now facing a future of great uncertainty. Our Association and our membership may be called upon for much greater contributions in the near future than in the past. With such hearty cooperation as has been

shown by our members in the past I have little fear but that they will give a good account of themselves whatever may come in the future."

President Cave then introduced Dr. W. E. Krauss, Associate in Dairy Nutrition Ohio Agricultural Experiment Station of Wooster, Ohio, who gave an address entitled, "Dairy Production Milestones," which will be found printed elsewhere in this issue of the Journal.

There were 298 members present. The meeting adjourned at 11:30.

GENERAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

Burlington, Vermont, June 26, 1941

President Cave called the meeting to order at 3:30 P.M. in the Fleming Museum, there being 124 present. Mr. Charles Blackman, chairman of the Necrology Committee, reported the death of the following members during the past year: Edward B. Meigs, A. R. Schubert, Elmer S. Hinman, Hugo Larsen, Clarence H. Redding, and Godfrey L. A. Ruehle. Information regarding the activities of these deceased members was contained in the report of the committee. Upon motion duly seconded the report was accepted to be made a matter of record in the minutes.

Editor Sutton then gave a report which will be found in the minutes of the board of directors.

MANUFACTURING SECTION

Secretary Anderson of the Manufacturing Section presented the following report:

The manufacturing section held its meetings at the scheduled hours and places. Mr. C. D. Dahle, chairman, presided.

All papers were presented as announced with the exception of "Production of Cream on Farms and in Plants," M 9, M 17, and M 30.

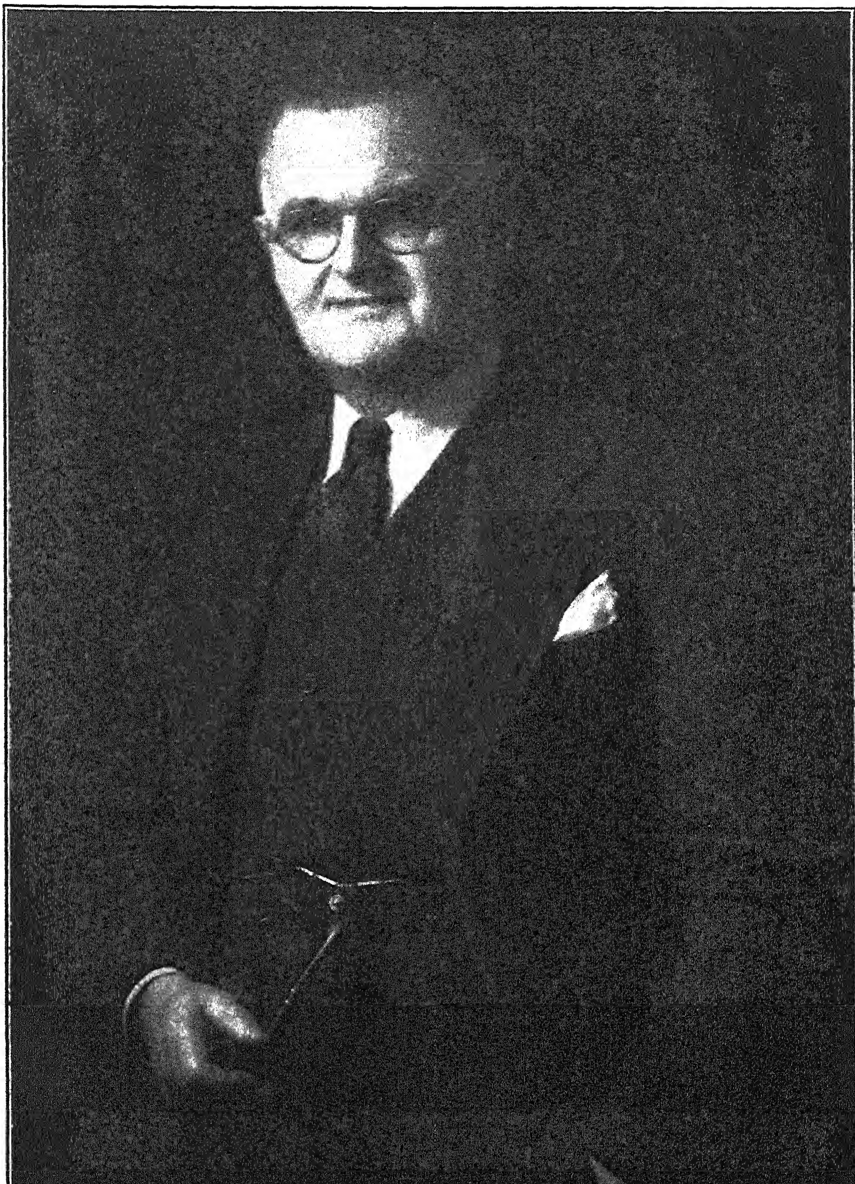
The business of the section was transacted at three meetings held at the announced places.

Reports were submitted by the various standing committees:

1. Committee on Chemical Methods for the Analysis of Milk and Dairy Products, L. C. THOMSON, *chairman*. The written report was accepted.

It was voted to appoint a special committee to compare the accuracy of the Gerber test with the Babcock test for milk and milk products.

It was voted that the chairman of the committee on the Chemical Analysis of Milk and Dairy Products act as coordinator in coordinating the work of said committee with like committees of other associations doing similar work. The report was accepted.



H. F. JUDKINS—PRESIDENT ELECT

2. Committee on Quality of Milk and Milk Products. W. V. PRICE in the absence of W. H. E. REID, *chairman,* called for sub-committee reports.

a. Cream quality—P. A. DOWNS, *chairman;* written report accepted.

b. Cream and butter quality—W. H. Brown, *chairman;* written report accepted.

c. Ice cream—no report.

d. Cheese quality—W. V. Price, *chairman;* written report accepted.

e. Market milk—F. C. Button reported for P. H. Tracy, *chairman;* written report accepted.

f. Condensed and milk powder—E. H. Parfitt, *chairman;* oral report accepted.

3. Committee on Students' National Contest in Judging of Dairy Products. G. M. Trout, *chairman;* written report accepted.

4. Committee on Methods of Determining the Curd Tension of Milk. F. J. Doan, *chairman;* written report accepted.

It was voted to accept the procedure for determining the curd tension of milk as recommended by the committee and that the committee be discharged.

5. Committee on Score Cards. C. J. Babcock, *chairman;* written report accepted.

It was voted to delete the flavor items under "remarks" on page 3 of the report and substitute words "flavor defects" listed on the other side.

It was voted that the score card for milk be approved as amended by the Manufacturing Section and submitted to the parent organization for final approval.

It was voted that the score card for ice cream be approved by the Manufacturing Section and submitted to the parent organization for final approval.

It was voted to accept the recommendations of the committee that cream be scored the same as milk until such time as a satisfactory score card can be devised.

It was voted that the committee remain active until it completes a score card for cream.

6. Committee To Study Methods for Measuring the Oxidation of Milk Fat. O. F. Garrett, *chairman;* written report accepted.

7. Committee on Methods of Measuring the Color of Milk. O. F. Garrett, *chairman;* written report accepted.

8. Committee to Study the Ways of Improving Summer Meetings of the Manufacturing Section. B. E. Horrall, *chairman;* written report accepted. It was voted that the report of the committee be accepted and that the committee be discharged.

It was suggested that the retiring manufacturing chairman carry over as a fourth member of the program committee.

It was voted to appoint a committee of three to work with the present chairman of the section to organize the present complex committee slate, to be effective October 1, 1941.

The following officers of the Manufacturing Section were unanimously elected:

Vice-Chairman—R. WHITAKER

Secretary—KENNETH G. WECKEL

Mr. L. H. Burgwald, present vice-chairman, automatically becomes chairman of the Manufacturing Section.

Respectfully submitted,

(Signed) E. O. ANDERSON

Secretary, Manufacturing Section

Upon motion duly seconded the report of the Manufacturing Section was accepted and ordered to be printed in the minutes. Copies of the written reports of the various committees of the Manufacturing Section were filed with the Secretary for their preservation.

EXTENSION SECTION

Mr. J. F. Kendrick, Secretary of the Extension Section presented the following report:

In session at the University of Vermont, Burlington, Vermont, June 24th, 25th, and 26th, 1941.

The annual meeting of the Extension Section was called to order by the chairman, Otto J. Hill, June 24 at 1:30 P.M. in Morril Hall. Forty-six members and 25 guests were present from a total of 25 different states.

During the three-day session the various committees of the Extension Section presented their reports supplemented with selected papers on pertinent phases of the dairy extension program.

The Sire Committee's report was presented by the committee chairman, E. J. Perry. The committee approved new age-conversion factor for use in Dairy Herd Improvement Associations; approved the use of Dairy Herd Improvement Association records in selective registry and Star Bull programs of the American Jersey Cattle Club; and recommended uniform methods of compiling annual reports of artificial breeding associations; also approved a bull leasing plan to combat the "stock-yard" bull problem.

Report was accepted.

The Feeding Committee report was presented by Mr. V. L. Gregg. This committee re-emphasized the importance of roughage feeding and outlined a tentative program to be followed by the Feeding Committee in succeeding years. The report was accepted as a progress report.

Quality Committee report presented by Mr. Evert Wallenfelt. The committee outline objectives of quality work to be discussed in future years. Report accepted as a progress report.

The Herd Health Committee reported to the joint session of the Extension and Production Section by Mr. C. G. Bradt. Report accepted as a progress report.

Report of the Joint Committee on Feeding Standards accepted.

Testing Committee report presented by Mr. C. R. Gearhart. The committee reported on standards for supervision of dairy herd-improvement association testing and recommended that further study be given the subject. The report was accepted as a progress report.

Type Classification Committee report given by Mr. J. W. Linn. The committee recommended type classification in herds where all cows are under test. Report accepted.

Exhibit Committee report presented by Mr. C. J. Fawcett. Exhibits illustrating extension methods used in 16 different States were presented.

Resolutions Committee report given by Mr. A. I. Mann, chairman of the Committee. The report was accepted and turned over to the General Resolutions Committee.

During the business session of the Section, Mr. E. C. Scheidenhelm of Michigan was elected Secretary. The officers who will assume their responsibilities on October 1, 1941, are as follows:

GLEN W. VERGERONT, *Chairman*
J. F. KENDRICK, *Vice-Chairman,* and
Chairman of the 1941 Program Committee
E. C. SCHEIDENHELM, *Secretary*

Respectfully submitted,

(Signed) J. F. KENDRICK
Secretary, Extension Section

Upon motion duly seconded the report was accepted and ordered to be printed in the minutes.

PRODUCTION SECTION

The Production Section held five regular scheduled sessions. Of these, two were symposia combined with the Extension Section and the Manufacturing Section respectively; a third was a Section symposium. The remaining two were devoted to regular papers and discussions grouped as to special subject matter. In accordance with the recommendation of the General Program Committee, this Section this year tested the plan of dividing the group into two divisions, with programs running concurrently. W. E. Petersen, Section Chairman, presided at sessions of Division A; H. A. Herman, Section Vice-Chairman, presided at sessions of Division B.

All sessions were well attended. Thirty-nine of the forty papers scheduled were presented.

The Section held three business meetings. Chairman W. E. Petersen presided at each meeting.

The minutes of the 1940 Annual Meeting of the Production Section at Lafayette, Indiana, were read and approved.

Reports of the various standing committees were submitted and ap-

proved. Copies of these reports are attached. Salient points incorporated in these reports and presented herewith to the General Session for approval are:

1. Breeds Relations Committee. W. T. Crandall (New York), *chairman.*

The revision of Rule 7 concerning the supervision of official tests will read: "Where the cows are milked by hand, only one cow may be milked at a time, if in a box stall or individual quarters. Two, however, may be milked at the same time, if milked by machine, or if standing in the stanchions in close proximity and in full view of the supervisor."

2. Committee on Measuring Results of Pasture Investigations. G. Bohstedt (Wisconsin), *chairman.*

This committee has continued its efforts of reconciling the viewpoints of workers engaged in pasture research. It is at present concerned with ways and means for printing their latest compilation of methods of pasture investigation technique.

3. Committee on Standard Methods of Analyses. W. E. Petersen (Minnesota), *chairman.*

Dr. Petersen stated that due to the large amount of material accumulated by the committee in the various phases of its study, it was deemed impractical to present the combined recommendations in a single report. The committee recommended that in view of the fact that methods of analyses by virtue of constant research were subject to frequent revisions and since the various fields had been carefully canvassed to date, it be discharged as a standing committee. It is further recommended that occasional papers be prepared for publication as reviews in the Journal of Dairy Science, on standard methods of analyses in individual areas of dairy production research.

4. Committee on Rules for Conduct of the Students' National Dairy Cattle Judging Contest. I. W. Rupel (Wisconsin), *chairman.*

5. Committee on Awards for Students' National Contest in Judging Dairy Cattle. A. A. Borland (Pennsylvania), *chairman.*

In addition to the regular awards that have been made at recent contests and that were repeated at the 1940 contest, two scholarships were secured. The Holstein scholarship of $500.00 was provided by Mr. Forry Laucks, owner of Lauxmont Farms, Wrightsville, Pa. The Ayrshire scholarship of $525.00 was provided by a group of prominent Ayrshire breeders in Pennsylvania.

6. The Feeds Specifications Committee. E. S. Savage (New York), *chairman.*

1—The American Dairy Science Association shall prepare through its Feeds Specifications Committee, an official table of analyses of feeds for dairy cattle. This table shall contain the usual percentages of water, min-

eral matter, protein and total digestible nutrients. A column showing the therms of net energy shall be given. The digestion coefficients shall be given as determined by cattle whenever possible. Whatever is available as to mineral and vitamin content shall be given.

2—This table shall be revised annually as new values appear.

3—Referees for different feeds shall be invited by the feeds specifications committee to study and report on single feeds or groups of feeds. These referees shall report annually on changes in analyses and new discoveries with respect to other qualities. Referees shall automatically become ex-officio members of the feeds specifications committee.

4—Efforts shall be made to refine feeding values of feeds by stimulating feeding experiments.

5—The members of the Feeds Specifications Committee shall cooperate fully with feed control officials and with similar committees of the American Society of Animal Production.

A change in the method of the appointment of committees was voted, whereby the incoming Section Chairman was delegated to make such appointments for the ensuing year. It was voted also that all new officers take office immediately following the close of the present annual meeting of the Association. It was felt that such a practice would enhance the formulating of the program for the following year's meeting.

By audible expression and show of hands, the Production Section went on record in hearty recommendation of the plan of program for the present meeting and recommended the continuation of such type of program.

Several excellent suggestions were made for symposia topics for future meetings.

Professor J. C. Knott, chairman of the nominating committee, presented names of candidates for offices of vice-chairman and secretary of the Section for 1941–42. K. L. Turk, Maryland, was elected vice-chairman; and Dwight Espe, Iowa, was elected secretary. H. A. Herman, Missouri, vice-chairman for 1940–41, automatically becomes chairman.

Respectfully submitted,

(Signed) K. S. Morrow

Secretary, Production Section

The following list of committees for the Production Section for 1941–42 was named by Section Chairman H. A. Herman and submitted to the secretary following the reading of the foregoing report:

1. *Breeds Relations Committee:*

F. W. Atkeson, Kansas, *Chairman* (1 year)
H. A. Herman, Missouri, *Secretary* (2 years)
Floyd Johnston, Iowa (1 year)
W. W. Yapp, Illinois (2 years)

J. B. Fitch, Minnesota (3 years)
E. C. Schiedenhelm, Michigan (3 years)

2. *Committee on Measuring Results of Pasture Investigations:*
   G. Bohstedt, Wisconsin, *Chairman*
   R. H. Lush, District of Columbia
   I. R. Jones, Oregon
   R. E. Hodgson, Washington
   C. B. Bender, New Jersey
   R. B. Becker, Florida

3. *Committee of Standard Methods of Analyses:*
   Committee excused.

4. *Committee on Rules for Conduct of Students' National Dairy Cattle Judging Contest:*
   I. W. Rupel, Wisconsin, *Chairman*
   S. M. Salisbury, Ohio
   J. R. Dice, North Dakota
   E. M. Hanson, Iowa
   P. M. Reaves, Virginia

5. *Committee on Awards for Students' National Contest in Judging Dairy Cattle:*
   A. A. Borland, Pennsylvania, *Chairman*
   Burt Oderkirk, Babson Co., Illinois
   G. E. Taylor, New Jersey
   I. W. Rupel, Wisconsin

6. *The Feeds Specifications Committee:*
   (From the Production Section)
   E. S. Savage, New York, *Chairman* (2 years)
   G. Bohstedt, Wisconsin (1 year)
   C. D. Grinnells, North Carolina (3 years)

   (From the Extension Section)
   C. L. Blackman, Ohio (2 years)
   W. T. Crandall, New York (1 year)
   M. J. Regan, Missouri (3 years)

   (Sub-committee on Digestion Coefficients)
   F. B. Morrison, Cornell, *Chairman*
   W. E. Krauss, Ohio
   S. Brody, Missouri

7. *Committee on Silage Methods, Evaluation, etc.:*

C. B. Bender, New Jersey, *Chairman*
T. E. Woodward (U.S.D.A.)
J. G. Archibald, Massachusetts
G. Bohstedt, Wisconsin
C. F. Monroe, Ohio
J. C. Knott, Washington State College

Upon motion duly seconded the report was accepted.

Mr. J. M. Frayer, Chairman of the Registration Committee, reported the attendance of 844 men, women, and children. Of the 492 men registered, 325 were active members and 167, non-members. These members represented 40 of the United States and Canada and the Philippines.

RESOLUTIONS COMMITTEE REPORT

Mr. K. S. Morrow, Chairman of the Resolution Committee, presented the following report:

The American Dairy Science Association assembled in its 36th Annual Meeting at the University of Vermont, wishes to express for the membership, their families and guests, its appreciation for the hospitality, delightful entertainment and splendid facilities provided by the officials and faculty of that University.

Therefore, be it *Resolved:* That the membership of the Association publicly express its most sincere appreciation to Dean and Director J. L. Hills; to Professor H. B. Ellenberger and his departmental staff; to the Ayrshire Breeders' Association, The American Guernsey Cattle Club, The Holstein-Friesian Association of America, and the American Jersey Cattle Club; to the several Vermont Maple Sugar producers and marketing organizations; and to all other agencies cooperating in the providing of entertainment and the many fine courtesies.

Whereas: The general health and physical well being of our people constitute the first essential in our national defense, and,

Whereas: The Selective Service Administration is finding an alarming proportion of our young men to be unfit for military service by reason of nutritional defects, and,

Whereas: Local, State and Federal health officials have long recognized the importance of an increased consumption of dairy products as a means of promoting national health and have encouraged and assisted the dairy industry in developing programs for the encouragement of increased dairy products consumption, and,

Whereas: There is an enormous potential supply of milk which can be developed whenever price and demand are sufficient to justify more liberal feeding of our dairy herds, and,

WHEREAS: The Government can always buy on the open market any needed supplies for shipment to Great Britain, and with this increased demand, effect an automatic adjustment of domestic supply and demand through resultant price changes without discouraging the desire for dairy products.

Therefore, be it *Resolved:* That this Association express its disapproval of any National program specifically designed to discourage the home consumption of dairy products or to develop in the minds of the consuming public the idea that dairy products are non-essential or unimportant in the National diet, which idea is in direct conflict with the long established and generally recognized recommendation of all public health and nutritional authorities, and,

Be it further *Resolved:* That a copy of this resolution be forwarded to the Honorable Secretary of Agriculture for his information.

WHEREAS: There has been a most valuable contribution to the National economy of this country through the educational and research activities of our Federal government, colleges and universities, and,

WHEREAS: The future welfare of the nation will depend to an even greater extent on activities of these agencies, and,

WHEREAS: The present emergency is necessitating the closest scrutiny and most conservative use of public funds and resources.

Therefore, be it *Resolved:* That this Association urge upon all public officials charged with the distribution of public funds, the importance of the continuance of an advancement of this program, and,

Be it further *Resolved:* That copies of this resolution be forwarded to the Honorable Secretary of Agriculture and all State Directors of Experiment Stations for their information.

WHEREAS: This American Dairy Science Association recognizes the valuable contributions made by the pioneer workers in doing research.

Therefore, be it *Resolved:* That this Association give public recognition to the excellent work developed by Dean J. L. Hills in the problem of experimental research methods and for his many other contributions to the field of Agriculture through his long years of valuable service as an instructor and Dean of Agriculture at the University of Vermont.

WHEREAS: The problems of herd health can best be attacked with the assistance and cooperation of all agencies and organizations concerned,

Therefore, be it *Resolved:* That a Herd Health Committee be appointed by the President of this Association to seek the cooperation of the American Veterinary Medical Association and the United States Live Stock Sanitary Board in formulating organized plans for an action program on these problems.

WHEREAS: This Association recognizes the importance of awards in giv-

ing incentive to students throughout the country for continued activity and study in the field of dairy cattle breeding and production,

Therefore, be it *Resolved:* That donors of prizes for the winners in the National Collegiate Students' Dairy Cattle Judging Contest be thanked individually in the name of the Production Section of the American Dairy Science Association by the Chairman of the Committee on Awards.

Be it *Resolved:* That the Dairy Cattle Breed Associations be commended for their constructive action in the organization of the Pure Bred Dairy Cattle Association of America, through which greater unification of plans and methods for the improvement of the various breeds may be attained.

WHEREAS: The Borden Company is continuing its awards for recognition of superior research in dairying.

Therefore, be it *Resolved:* That the American Dairy Science Association express its appreciation to the Borden Company for its continuing interest in dairying.

Respectfully submitted,
K. S. MORROW, *Chairman*
C. Y. CANNON
HAROLD MACY
E. C. SCHEIDENHELM
C. N. SHEPARDSON

Upon motion duly seconded the report was accepted.

NOMINATING COMMITTEE

Mr. Earl Weaver, Chairman of the Nominating Committee, submitted the following report:

For *Vice-President:* H. P. DAVIS; JAMES W. LINN
For *Director* to succeed M. E. Parker: L. S. PALMER; G. M. TROUT
For *Director* to succeed J. W. Linn: J. C. KNOTT; L. P. LAMASTER

Committee:
EARL WEAVER, *Chairman*
R. B. BECKER
R. R. GRAVES
J. B. FITCH
D. R. THEOPHILUS

Upon motion duly seconded the report was accepted.

SECRETARY-TREASURER'S REPORT

The Secretary-Treasurer then gave the following report:

The policy of the American Dairy Science Association in conducting their business has been changed in recent years, and at present the Board of Directors, who are elected for a period of three years by ballot, conduct

the business of your Association. It is their wish, however, that a summary of their action be presented to the membership so that the members present at this annual meeting may know as early as possible the action that has been taken by the Board of Directors.

A copy of the Certified Public Accountant's Audit was made February 1, 1941, and mailed to each member of the Board of Directors.

Although you may not be interested in a detailed report, you will be interested in learning that our income last year was $17,347.00 and our operating expenses were $16,542.00. Our net worth is $18,919.55.

Our increased expenditures are largely due to increased cost of our Journal. Up until 1933 our Journals were limited to 550 pages per volume. In 1932 the style was changed so that each page contained an equivalent of one and one-third pages. In 1937 the volume of the Journal contained over 1100 pages which was twice as many pages as the volumes which had been published previous to 1934. Last year our Journal contained 1662 pages or more than three times the previous limit. When one takes into account both the style change and increased number of pages, the XXIII Volume (1940) contained four times as many words as did the volumes previous to 1932.

Our circulation reached 2438 in 1939 and 2406 in 1940, but on June 17 of this year we had 129 greater circulation than at the same time last year which would indicate that our circulation will exceed 2500 this year. This is 50 per cent higher circulation than in 1936 when it was 1652.

Our increased circulation this year is largely due to an increased number of student affiliates. Last year we had a total of 282. At this same date last year we had 242. We now have 368 which is an increase of 126 over last year. Of the 368 student affiliates, Iowa leads with 62. Ohio is second with 43; Wisconsin third with 26; Massachusetts fourth with 22; Illinois fifth with 17. Other states with ten or more student affiliates are Texas, Vermont, Pennsylvania, Indiana, Michigan, Missouri, New York, South Carolina, Virginia and the State of Washington.

New Members

We may expect to lose about 7 per cent of our membership each year by death, resignations or one thing or another. It is therefore essential that each state have new members to the extent of about 7 per cent of their membership to prevent a decreased number of members.

Up to this date this year we have 83 new members; 31 of whom were student affiliates and 52 of whom have paid the $5.00 affiliation fee. Illinois and Pennsylvania lead with 7 new paid members each; Oklahoma, Vermont, and New York tie for third place with 4 each; and Massachusetts, Ohio, and Wisconsin tie for sixth place with 3 each.

Permit me to say a word about the affiliation fee. Some of our members

may not see the need of charging this fee. Our net worth, most of which is invested in United States Government Securities, amounts to $18,919.55. If our 1400 members were to share this equally, each one of us would receive $13.51. It is therefore only proper that these new members who have not contributed anything to this accumulation should pay $5.00 or less than one-half of a membership's value into this fund.

This does not apply to student affiliates. They not only are permitted to receive the Journal at $3.00 per year, which is less than our cost of printing the Journal, but upon finishing school, they are eligible to become full members by merely paying their $5.00 dues. In 1939 we had 156 student affiliates; in 1940, 282; and this year we have 368 student affiliates.

Back Copies

The Association is now prepared to furnish to any of its members or any library a complete file of all back numbers and volumes. We suggest that you check your library and complete your volumes of the JOURNAL OF DAIRY SCIENCE. As soon as the twenty-year index is published the first twenty volumes will be a very valuable reference for your office and library. You will find the price list for back copies printed in the advertising section of the Journal.

Advertisers

We are grateful for the commercial companies that use our Journal as an advertising medium. Last year income for advertising amounted to over $4,000, which is equivalent to the dues of 800 members. Any courtesies shown these advertisers will be appreciated.

Reprints

We recommend that those of you who are in charge of having bulletins printed that you investigate purchasing reprints through our Printers of all articles published in the JOURNAL OF DAIRY SCIENCE. We are of the opinion that the reprints will be furnished you at a lower cost than you will be able to have them printed, and the Association gets a small income from all reprints sold.

The Secretary then reported on all the action taken by the Board of Directors. Motion was made, duly seconded and passed that the Minutes of the Board of Directors be accepted and the Association approve and endorse all action that the Board of Directors had taken during the past year.

MEETING OF BOARD OF DIRECTORS AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, *Secretary-Treasurer*

Burlington, Vermont, 9:30 A.M.*, June 23, 1941*

A meeting of the Board of Directors of the American Dairy Science Association was held in the Delta Delta Delta House, Monday, June 23, 1941, at 9:30 A.M.

Present: President H. W. Cave; Vice-President H. F. Judkins; Secretary-Treasurer R. B. Stoltz; Directors, J. W. Linn, M. E. Parker, C. N. Shepardson, Fordyce Ely, H. B. Ellenberger, A. C. Dahlberg, E. S. Guthrie.

Editor T. S. Sutton then presented the following report:

EDITOR'S REPORT

The Editor begs to submit the following brief report to the Board of Directors of the Association:

1. *Summary of Journal Contents.*

A summary of the Journal contents over the past three years is presented in the accompanying table.

SUMMARY OF JOURNAL CONTENTS

	1938–39	*1939–40*	*1940–41*
Number of original articles	88	94	97
Pages of original articles	760	826	892
Number of reviews	2	3	5
Pages of reviews	26	119	144
Miscellaneous	140	98	157
Students National Contest, Proceedings Annual Meeting, Announcements, Circulation, Index, Committee reports.			
Pages of Abstracts	232	206	304
Total number of pages printed	1158	1245	1497
Classification of Articles			
Manufacturing articles	52	53	56
Pages occupied by Manufacturing	482	450	514
Production articles	28	31	35
Pages occupied by Production	212	296	316
Manufacturing-Production	8	10	6
Pages occupied	66	80	62
Classification of Reviews			
Manufacturing Reviews			5
Pages occupied by Manufacturing Reviews			144

2. *Abstracts.*

You will note a substantial increase in the number of pages of abstracts

during the past year. We believe also that the quality of the work done has been improved. Steps have recently been taken to make further improvements particularly in reference to uniformity in the citation.

3. *Style Standard.*

The committee on Style Standard has made their report to the Editor and Journal Management Committee. A "Note to Contributors" has been prepared which we trust will be mutually helpful to author and editor. It is intended that this shall be printed on one Journal page in small type and regularly carried in the Journal.

Again we want to take this opportunity to publicly express our gratitude to all those who have given so generously of their time and energies in our assistance. To them is due the credit for any measure of success attained.

The following action was taken by the Board of Directors:

1. The Editor's report was accepted.
2. The Secretary was instructed not to publish a list of the members in the December Journal.
3. The budget was made for a period of 18 months.
4. Student branches were authorized at Cornell and Oklahoma.
5. From the report of the Committee on Journal Management:
   - A. The twenty-year index is to be sold at cost, which is approximately \$4.50. Pre-publication price to members is to be \$1.00; after-publication price, \$2.00. The Secretary was authorized to place the pre-publication price on annual statements.
   - B. Approve policy adopted last year in paying for abstracts.
   - C. Abstracts of papers presented at annual meeting are to be published in the August Journal.
   - D. The report of the Committee on Style Standards was adopted and ordered printed.
   - E. The pages per volume of the Journal should not exceed the present number.
6. Report of Auditing Committee was accepted.
7. The Secretary-Treasurer was authorized to send a copy of the minutes of the Board of Directors to each officer and director, and to condense the minutes that are to be printed into about 15 pages. He was further authorized to make a very brief report of all board action at the General Business Session.
8. The following report of the Committee on Divisions was accepted:
   - A. That each of the three divisions be paid \$25.00 upon receipt of a report of their meeting, and the names of the elected officers for the ensuing year.

B. That the divisions hold their election by ballot of those members who attend the divisional meetings.
9. That the $1,025 collected by Professor Borland be turned over to the Chairman of the Awards Committee of the Production Section when requested.
10. Texas was selected for the 1943 meeting.
11. The tentative program of the next annual meeting is to be printed separate and distributed with the May issue of the Journal.

AMERICAN DAIRY SCIENCE ASSOCIATION PRESENTED BORDEN AWARDS

TO

P. F. SHARP

AND

E. B. HART

University of Vermont

Burlington, Vermont, June 26, 1941

Mr. H. B. Ellenberger acted as toastmaster at the Annual Association Banquet, and presented Gov. William H. Wills of the State of Vermont, Dean J. L. Hills of the University of Vermont's Agricultural College, and Mr. W. D. Dotterer, acting chairman of the Committee for the Borden Award in Dairy Manufactures.

Mr. Dotterer made the following statement:

"Corporations are not always the soulless organizations they have been accused of being. There are times when they show more humanitarian characteristics than some of the individuals who make the unwarranted accusations. This meeting is the result of far-sighted scientific appreciation by one of the large corporations engaged in the dairy business. They are under no obligation to industry or to the universities to provide a prize for outstanding work in dairy investigation or development, but they have generously provided two $1,000 awards for such effort. May I take this opportunity to express the appreciation of the American Dairy Science Association to the Borden Company for this generosity in providing this very substantial gift to one of our great scientists.

"I do not suppose that any of the scientists engaged in dairy research have been influenced in the quality or quantity of their work by the thought of the Borden Award. Rather they pursue their labors with the object of learning the truth and making their work available to their colleagues and to the whole industry. If this statement seems doubtful, one need only note the number of new and improved processes which have been given to any who would use them for the benefit of humanity.

PAUL FRANCIS SHARP

"The man chosen to receive the award in dairy manufacture this year is well known to every one interested in the scientific aspects of dairying. He has been the inspiration and leader in a great number of important

researches. He is recognized as one of the best dairy scientists in the world. He is one of the quiet, unassuming, gentlemanly personalities whom it is a delight to know and to work with. His statements are carefully made and can be taken for facts. All I have said can be applied to many other investigators in the dairy field. In fact, the first impression made on the Committee when the data on so many men was presented for consideration was that a choice would be well night impossible. However, after much deliberation, a choice has been made. The Committee hopes and believes that out of the galaxy of stars a selection has been made with which you will agree. We know the successful nominee is worthy of the prize and only regret that more prizes were not available.

"It is not my duty to make a speech but to name the recipient of the Borden Award in Dairy Manufactures for 1941. He is Paul Francis Sharp of Cornell University. Dr. Sharp received his Bachelor of Arts degree at Nebraska Wesleyan University in 1917, his Master of Science at the University of Minnesota in 1920 and his Doctor of Philosophy at the University of Minnesota in 1922. He was student assistant in Chemistry at Nebraska Wesleyan and the University of Minnesota 1917–1922; also in chemical warfare service, U.S.A., 1918. He was associate chemist at Montana Agricultural Experiment Station 1922–25. He has been Professor of Dairy Chemistry and chemist in the Agricultural Experiment Station of Cornell University since 1925.

"Dr. Sharp is one of those tireless workers who have given so much to the Dairy Industry. His publications are legion and the information he has furnished has been of inestimable value commercially. It is not easy to say which of his accomplishments have been of the greatest use to dairying and to humanity. Studies on the lipolytic activity of milk in relation to flavor, studies on Vitamin C, studies on oxidized flavor, studies on the physical state of milk fat and studies on the de-aeration of milk are some of the projects which have helped to make him so well known.

"The Committee unanimously chose him as the most deserving and it is my privilege to present to you, Mr. Wentworth, Dr. Paul Francis Sharp, in order that you may give him the really substantial part of the award."

Mr. Sharp came to the platform, and Mr. W. A. Wentworth of the Borden Company presented Mr. Sharp a gold medal and a check for $1,000.

Mr. Ellenberger, the toastmaster, then introduced Mr. G. C. White, acting chairman of the Committee for Borden Award for Production. Mr. White then made the following statement:

"The three members of the Production Award Committee, whose duty it was to select the recipient of the 1941 Borden Award for outstanding research in dairy production, have unanimously chosen Professor Edwin Bret Hart of the Wisconsin College of Agriculture for this honor. Professor

Hart's numerous contributions to our scientific knowledge and his service to the dairy industry over the last 40 years mark him as the outstanding candidate for this award.

"Professor Hart was born December 25, 1874, at Sandusky, Ohio. He received his B.S. degree from Michigan in 1897 and later attended the Universities of Heidelberg, and Marburg, Germany, in 1900–1901. His first

EDWIN BRET HART

position in this country was with Dr. L. L. Van Slyke of the New York Experiment Station at Geneva, where he was assistant chemist from 1897 to 1902 and associate chemist from 1902 to 1906. He was appointed Professor of Biochemistry and Chairman of the Department of Biochemistry at the University of Wisconsin in 1906, which positions he holds today.

"Among the numerous meritorious contributions to fundamental dairy knowledge which the Committee found in Professor Hart's record, the following are cited as deserving of special mention:

"(1) The determination of phosphorus in feeds and the rôle of phosphorus in nutrition of animals. Also his work on the chemical changes which take place in ripening cheese. This work is the most complete and significant that has ever been done on this subject.

"(2) The relationship of copper and iron for building blood hemoglobin, in the prevention or cure of nutritional anemia.

"(3) The importance of minerals other than iron and copper in animal nutrition, especially phosphorus and its availability from both organic and inorganic sources; iodine in the prevention of goitre, referred to as 'big neck' in ruminants; and magnesium as supplied by dolomitic limestone, which is the prevailing limestone in many parts of the country.

"(4) A fuller understanding of the function of protein in dairy and livestock nutrition, the supplementary relationships of proteins from different plant and animal sources, and the place of simple forms of nitrogen such as urea and ammonium compounds as sources for protein building. Few research men in any country have done more effective work than Professor Hart in the protein nutrition of dairy cattle.

"(5) The existence of the 'grass juice factor' in animal nutrition, which has particular reference to summer milk, and to winter milk which has been produced on superior roughages.

"(6) The favorable effect of fat on the utilization of lactose in milk.

"(7) The superior value of butterfat over vegetable oils, through virtue of certain essential fatty acids.

"Last, but not the least, the Committee wishes to call especial attention to Professor Hart's leadership in training scientists. Many brilliant young men have sought an opportunity to work with Professor Hart in his laboratory at Madison. Many of these Hart-trained men are now leaders in many experiment stations and in industry."

In the absence of the recipient, Mr. Gus Bohstedt of the University of Wisconsin, was called to the platform. Mr. W. A. Wentworth of the Borden Company presented Mr. Bohstedt the gold medal and a check for $1,000 and requested him to carry it to his colleague, Professor E. B. Hart.

Mr. Wentworth then gave a summary of the awards granted during the past five years and assured the Association of their continuation for at least one more year.

JOURNAL OF DAIRY SCIENCE

VOLUME XXIV | SEPTEMBER, 1941 | NUMBER 9

COWS' URINE AS A FERTILIZER FOR BLUEGRASS PASTURES

W. B. NEVENS

Illinois Agricultural Experiment Station, Urbana, Illinois

Most dairy farmers recognize the fact that dairy cows' urine has some value as fertilizer, but as a rule, its true value is not appreciated and it is not as well conserved and utilized in crop production as the solid portion of the excreta. The object of the investigation reported herewith was to call attention to the high value of cows' urine as a fertilizer by demonstrating its effects upon bluegrass pasture.

The urine of dairy cows normally contains one-third to one-half of the nitrogen and three-fourths or more of the potassium in the excreta (feces and urine) of these animals. As much as 12 to 16 pounds of nitrogen and 10 to 12 pounds of potassium may be found in the urine for every ton of feces excreted (6).

Only a few reports of experimental studies of the value of urine as a fertilizer for pastures are to be found in the literature.

Curtiss (1) reported that the application to bluegrass pasture of 4000 pounds of urine containing .7 per cent nitrogen, .167 per cent potash, and .033 per cent phosphorus, increased the yield of grass 26.5 per cent, or equivalent to 650 pounds of hay per acre.

Ernest (2) states that "Danish experiments with urine have shown that the effect of an autumn application was only 30 to 40 per cent that of a spring application." (Abstract taken from Pieters (8).)

Falke (3) found that the effect of fertilizers on naturally established pastures was to increase "the percentage of protein as well as the digestibility of the protein except that Plot 8 which received urine rather than nitrate of soda fell below the others in production." (Abstract taken from Pieters (8).)

Zacharewiez (10) reported that the use of liquid manure, superphosphate, complete chemical fertilizer, and stable manure, increased the yields of a meadow 17 per cent, 48 per cent, 78 per cent, and 69 per cent, respectively, based upon the yields of check plots.

Received for publication April 1, 1941.

EXPERIMENTAL METHODS

Four plots, each 2 × 2 rods in size, were laid out in a well-sodded and nearly level part of a 4.5-acre bluegrass pasture. Short posts were set at the corners of each plot and the plots were separated from each other by 1-rod borders. No fencing other than the posts was used so that the cattle grazing in the pasture had free access to all of the plots. Reinforced wire cages approximately 4′ × 4′ in size were used to protect sampling areas within each of the plots (Fig. 1). Cages were also placed in the pasture nearby to facilitate the sampling of a control area.

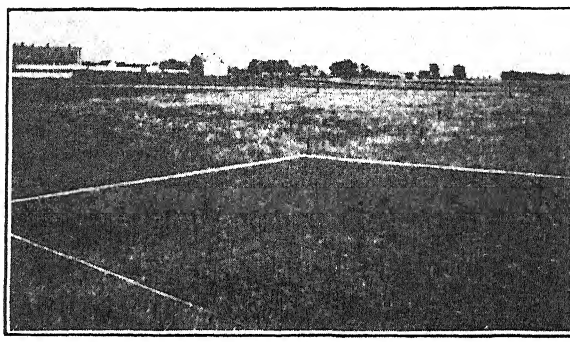


FIG. 1. The fertilized plots (marked by short posts) were grazed closely while the grass on the unfertilized portions of the pasture headed out. Plot 4 (marked by white lines) is in the immediate foreground. Plot 1 in the background shows but little more intensive grazing than the unfertilized pasture. Photographed July 4, 1939.

The first samples of the season were harvested immediately prior to turning the cattle to pasture. Only one area on each plot was harvested on the first sampling date. During the taking of the first samples, two wire cages were placed at each sampling location. One of these was placed over an area just harvested. The forage taken from this area the following month comprised only the forage produced by one month's growth. This was designated the "A" sample.

On each sampling date a cage was placed over a representative portion of the open pasture. The forage harvested from this protected area the following month formed the "B" sample. It comprised not only forage produced during the one month's growth, but also the forage on the area at the time the protecting cage was put in place the previous month. Hence, in computing the yields by the "B" method, the amounts of forage on the open pasture the month previously as determined from the "C" samples were subtracted from the "B" samples.

The "C" samples consisted of harvests of forage from the open, or unprotected, portion of each plot on each harvest date. These samples indicate

only the amounts of forage on the pasture at the time of harvest and used alone do not represent yields.

The samples were harvested by the use of a metal frame and grass shears (7). The metal frame was 44″ × 44″ in size and 1½″ high. It was braced by cross rods. A flat sliding bar laid over the top formed a guide for the shears to insure cutting of the forage at the same height each time.

The forage was collected in cloth sacks and taken to the laboratory where it was at once separated by careful hand sorting into weed and grass portions. Each portion was resacked in tared cloth sacks, weighed, and dried in a constant-temperature electrically-heated oven at 95°–100° C. The grass portion, which consisted almost entirely of Kentucky bluegrass (*Poa pratensis*), was analyzed for its nitrogen content.

The urine was collected from high-producing dairy cows into clean pails during the act of urination. It was applied by hand within a few hours after collection by means of garden sprinkling cans. In most cases it was applied undiluted. Because of a low moisture content in the surface soil, the urine applied May 13, 1939, was mixed with an equal volume of water with the object of preventing injury to the grass.

Applications of urine were made in April, May, and June of 1939, and in May and June of 1940. The rates of application in pounds per acre to Plots 1, 2, 3, 4, and 5 were 1250 pounds, 2500 pounds, 3750 pounds, 5000 pounds, and 0 pounds, respectively. The first treatment in 1940 was delayed until after samples of the forage had been taken, in order to determine if there was a carryover effect of the previous year's treatment. The nitrogen content of the different lots of urine ranged from 1.068 per cent to 1.29 per cent and the potassium content from 0.91 per cent to 1.22 per cent.

EXPERIMENTAL RESULTS

Heavy applications of nitrogenous fertilizers to grasses sometimes cause "burning," an injury which may temporarily retard growth or in some instances completely kill the plants. No burning, or injury, of the grass was noted following the application of urine except after the treatment made June 8, 1940. In spite of apparently ample moisture in the soil from a recent rain, some burning of the grass occurred in the two most heavily fertilized plots, especially on the small areas from which samples had been harvested two days before. The urine aplied on that date carried more than 1 per cent nitrogen and approximately the same percentage of potassium, or slightly more than 20 pounds of each of these elements per ton of urine. The applications were such that larger quantities of nitrogen and potassium were applied annually than is customary in the use of commercial fertilizers.

Striking differences were found in the protein content of the grass harvested from the various plots (tables 1 and 2). Several generalizations may be drawn from the data, viz.:

TABLE 1

Composition of samples harvested from bluegrass pasture plots in 1939

Plot No.	Date of harvesting samples									
	May 5		June 7		July 5		Aug. 8		Sept. 11	
	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter
	per cent	*cent per*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
				"A" Samples						
1	36.0	18.4	40.3	12.5	33.3	14.8	35.7	14.4	40.6	14.6
2	36.5	19.2	44.3	14.1	*	*	....	14.8	....	15.6
3	32.1	21.2	37.4	18.7	31.7	16.4	26.3	17.4	35.7	16.1
4	32.7	21.6	40.7	16.3	34.7	18.6	36.4	19.1	39.0	16.6
5	30.8	16.7	38.9	10.6	36.5	12.6	33.3	15.0	38.0	16.7
				"B" Samples						
1	36.0	18.4	41.7	11.0	44.5	10.8	50.0	10.3	43.3	11.3
2	36.5	19.2	47.7	10.8	39.1	12.5	41.0	11.6	39.5	13.9
3	32.1	21.2	39.3	14.8	34.8	14.7	43.1	12.5	41.2	16.8
4	32.7	21.6	43.7	16.5	35.4	18.3	40.4	15.1	39.3	16.9
5	30.8	16.7	44.2	9.5	42.0	10.2	49.4	10.1	41.5	13.8

* Cage moved by cattle; no sample.

TABLE 2

Composition of samples harvested from bluegrass pasture plots in 1940

Plot No.	Date of harvesting samples									
	May 2		June 6		July 5		Aug. 12		Sept. 26	
	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter	Dry matter content	Protein in dry matter
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
				"A" Samples						
1	25.0	17.7	29.8	11.9	31.5	13.4	34.1	12.6	33.3	18.6
2	25.0	27.0	27.5	12.0	26.4	16.1	30.8	12.7	35.3	21.6
3	17.6	19.6	29.1	14.1	29.1	17.5	33.3	13.3	33.3	19.4
4	21.2	18.4	27.8	13.7	31.7	17.6	27.3	12.8	35.7	23.8
5	24.4	16.8	24.1	10.5	37.0	12.1	....	11.0	37.5	17.4
				"B" Samples						
1	25.0	17.7	31.1	9.0	36.9	10.2	48.1	8.6	52.0	12.7
2	25.0	27.0	29.4	10.9	37.1	10.9	45.4	9.4	50.0	*
3	17.6	19.6	29.1	11.8	35.5	16.1	48.2	8.8	42.9	17.1
4	21.2	18.4	30.9	11.1	37.1	13.3	45.9	13.4	40.0	17.4
5	24.4	16.8	33.3	8.3	44.7	7.4	36.6	9.4	54.5	13.4

* Sample lost.

(a) In most instances the protein content of the grass on the treated plots was higher than that of the untreated, and the larger the amount of urine applied, the higher the protein content. Exceptions occurred during nearly dormant conditions of the pasture in August and September of both years, and in several instances the protein content of Plot 3 was higher than that of Plot 4.

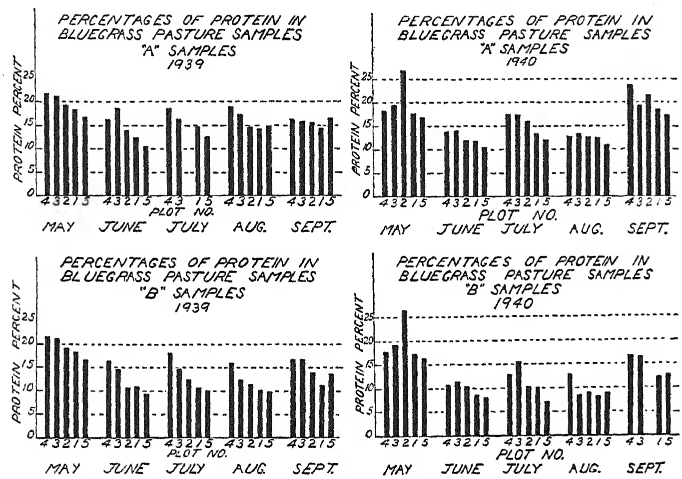


FIG. 2. Treatment of bluegrass pasture plots with cows' urine increased the protein content of the grass over that of the control area (Plot 5), and during the early part of the season, the heavier the application, the higher the protein content of the grass.

(b) The protein content of the A samples was higher than that of the B samples. This is attributed to the fact that the A samples represented only new growth, while the B samples comprised both new growth and older forage. In comparing the analyses of the A and B samples given in tables 1 and 2, it should be noted that the analyses of the samples harvested May 5, 1939, and May 2, 1940, have been listed under both A and B samples in order to facilitate comparison of the A and B samples with the first samples of the season. Following the terminology used in this report, these first samples were neither A nor B samples, but were C samples.

(c) The protein content declined rapidly with advancing development of the plants and dry weather. Rainfall during the summer months of 1940 was less than during the corresponding period of 1939 (table 3) and the protein content of the samples harvested in the summer of 1940 was somewhat less. Light showers during the latter part of August, and in September, 1940, stimulated some new growth with a consequent rise in the protein content of the harvest made Sept. 26, 1940. In this experiment a renewed growth induced by rain seems to have been fully as potent a factor or an even more important factor than fertilization in enhancing the protein content of the grass.

TABLE 3

*Rainfall at Urbana, Illinois**

Month	Average 1889–1940 incl.	1939	1940
	inches	*inches*	*inches*
April	3.53	5.39	3.96
May	3.87	1.19	4.53
June	3.73	6.17	5.04
July	3.08	1.73	0.95
August	3.34	6.38	2.80
September	3.22	0.32	0.48
October	2.43	2.54	1.93
Total for year	35.03	38.05	30.60

* University of Illinois Cooperative Weather Bureau.

(d) There was a carryover effect of the urine treatments of 1939 which lasted not only throughout the sampling period but was also evident in the samples harvested May 2, 1940. The unusually high protein content found for Plot 2 on May 2, 1940, is unexplained even after a repetition of the analysis. However, even after leaving out of consideration this unusually high figure, the protein content of the grass from the other three plots was found to be substantially higher than that of Plot 5, the control area. As pointed out above, the first application of urine in 1940 was not made until after these samples had been harvested.

TABLE 4

Yields of dry matter and amounts of dry matter in open pasture on bluegrass pasture plots in 1939

Method of determination	Amounts per acre of dry matter				
	Plot No.				
	1	2	3	4	5
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
"A" grass	3143	3095	3435	4310	2317
"A" weeds	161	38	308	292	259
Total	3304	3133	3743	4602	2576
"B" grass	4083	3110	3857	3984	2771
"B" weeds	162	26	614	16	49
Total	4245	3136	4471	4000	2820
"C" grass	3726	2511	2170	1685	4519
"C" weeds	16	6	114	97	32
Total	3742	2517	2284	1782	4551

The yields of dry matter in the forage are shown in tables 4 and 5. The yields as determined by the A method, or the harvests of the new growth (tables 4 and 5), were larger for the treated plots (Nos. 1–4) than for the control plot (No. 5). Also, the June and July harvests of 1939 indicated that the larger the application of urine the larger the yield of dry matter in

the bluegrass. Low rainfall in July and also during the latter part of the pasture season of both years was followed by such low yields that no direct relation between method of treatment and yield was apparent.

TABLE 5

Yields of dry matter and amounts of dry matter in open pasture on bluegrass pasture plots in 1940

Method of determination	Amounts per acre of dry matter				
	Plot No.				
	1	2	3	4	5
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
''A'' grass	3402	3694	2980	3759	2041
''A'' weeds	65	97	567	486	97
Total	3467	3791	3547	4245	2138
''B'' grass	3013	4180	4018	2268	1103
''B'' weeds	0	97	728	339	470
Total	3013	4277	4746	2607	1573
''C'' grass	4309	3564	1684	3531	3790
''C'' weeds	97	65	113	292	48
Total	4406	3629	1797	3823	3838

The yields as determined by the B method (*i.e.*, B samples—C samples of previous month) are for the most part in substantial agreement with those determined by the A method, particularly with respect to the higher yields of the treated plots than of the untreated. Considering the small size of the areas harvested in sampling, the agreement of the two methods seems remarkably good. The merits of these methods of determining yields have been discussed by Fuelleman and Burlison of this Station (4, 5).

Plots 1–4 were nearly bare from July to September, 1939, and during this period the amounts per acre of forage on these plots were less than on Plot 5, the control plot. Probably this closely grazed condition of the treated plots in the fall of 1939 accounted for smaller yields from them than from Plot 5 in May, 1940. A summary of the yields for 1940 is given in table 5. In spite of low yields of Plots 1–4 in May, 1940, the yields of these plots for the season, as determined by both the A and the B methods, were much higher than for the check plot.

The palatability of the grass on the urine-treated plots was apparently greater than that of the untreated portions of the pasture. Within a few weeks after the cattle were first turned to pasture in 1939, it was evident that Plot 4 and a little later Plot 3 were being grazed more heavily than the other plots, or the untreated pasture. A short time after, Plot 2, and finally Plot 1, were given more attention by the cattle. On the treated plots the grass was grazed closely except around droppings, while on the borders between the plots and the rest of the pasture the grass headed out (Fig. 1). It appears from the data that there was a direct relationship between the

protein content and the palatability of the bluegrass, *i.e.*, the higher the protein content, the greater the palatability.

SUMMARY AND CONCLUSIONS

Four small plots of Kentucky bluegrass were treated in April, May, and June of 1939, and again in May and June of 1940, with applications of cows' urine at rates ranging from 1250 pounds to 5000 pounds per acre at each application. A control area was untreated. Samples of the grass were harvested monthly from May to September, inclusive.

Although the urine contained more than 1 per cent nitrogen and in most cases more than 1 per cent potassium, the heavy applications were, as a rule, not harmful to the forage.

The protein content of the grass on the treated plots was higher than that of the grass on the control area, and in most instances, the heavier the application of urine, the higher the protein content.

The protein content of the A samples, representing recent growth, was higher than that of the B samples, which included both older forage and recent growth. Advancing development of the plants and renewed growth induced by rains were important factors affecting the protein content of the grass, the former causing a decline and the latter an increase in protein content.

The effect of the first year's spring treatment with urine on the protein content of the grass was evident during the remainder of the pasture season and also in May of the following year.

The yields of the urine-treated plots were higher than that of the untreated pasture and there was a tendency toward higher yields from the more heavily treated plots.

The palatability of the grass, as evidenced by close grazing by cattle, was higher on the urine-treated plots than on the untreated pasture and the greater the protein content of the grass, the greater the intensity of grazing.

ACKNOWLEDGMENT

This paper constitutes a report of an investigation of pasture improvement methods conducted cooperatively by the Departments of Agronomy, Animal Husbandry, and Dairy Husbandry, University of Illinois. The assistance and counsel of members of these Departments is hereby gratefully acknowledged. The chemical analyses reported were made under the direction of Mr. J. M. Lindgren of the Applied Chemistry Testing Laboratory of the University of Illinois.

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RANCIDITY STUDIES ON MIXTURES OF RAW AND PASTEURIZED HOMOGENIZED MILK*

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When raw milk is homogenized there is an immediate and continued rise in the titratable acidity (1, 4), accompanied by the development of a rancid flavor. Pasteurization of the milk prevents rancidity from developing. The phenomenal development of rancidity in homogenized raw milk has been attributed to the action of lipase normally present in all milk, through activation of the lipase itself, through the creation of new surfaces more susceptible to lipase action, or through the increased surface area of the fat globules. This lipolytic response to the homogenization of raw milk is recognized by the market milk industry to the extent that pasteurization is a closely allied process to the homogenization of milk for bottling purposes.

Even though pasteurized milk will not develop rancidity upon homogenization, nevertheless, information is available indicating that rancidity may be induced in this pasteurized milk by the addition of raw homogenized milk. For example, Dorner and Widmer (2) stated that "mixtures of pasteurized and homogenized cream or milk with raw milk, raw skim milk, or raw cream become rancid." Gould and Trout (3) have demonstrated that in mixtures of homogenized raw and unhomogenized pasteurized milk lipolysis proceeded to a greater extent than if the fat splitting had been calculated as having occurred only in the raw product.

In the study herein presented combinations of homogenized and unhomogenized raw and homogenized and unhomogenized pasteurized milk were made to ascertain under what conditions and to what extent rancidity would occur.

EXPERIMENTAL PROCEDURE

Raw milk was secured from the College milk supply which was composed largely of mixed milk from herds of several producers as well as that from the College herd. Lots of the milk were prepared which consisted of unhomogenized raw, homogenized raw, unhomogenized pasteurized and homogenized pasteurized milk. Homogenization was at 2500 pounds pressure, with the milk at approximately 100° F. in the case of the raw milk and at the pasteurization temperature in the case of the pasteurized milk. Pasteurization was conducted at 145° F. for thirty minutes.

The following mixtures of milk were prepared: (a) unhomogenized raw milk with homogenized pasteurized milk at a rate so that the samples con-

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tained 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, and 100 per cent of raw milk; (b) homogenized raw milk with homogenized pasteurized milk in the same proportions as stated above; and (c) unhomogenized raw milk with homogenized raw milk in the same ratios as in (a) and (b). These samples were titrated for increases in acidity, conveniently expressed as lactic acid, with 0.05N NaOH, and were studied organoleptically for the development of rancid flavor immediately after processing and preparing the various mixtures and after 1, 3, 7 and 10 days of storage at 35° to 40° F. The increase in titratable acidity was determined by subtracting the acidity of the unprocessed raw milk after the various storage periods from the titratable acidity of the mixtures after similar storage. The degree of rancidity was expressed numerically as follows: 0, no rancidity; 1, questionable; 2, slightly rancid; 3, distinctly rancid; 4, pronounced rancid. The numerically averaged flavor scores represent the average scores of two or more judges.

EXPERIMENTAL RESULTS

Lipolytic activity in mixtures of unhomogenized raw and homogenized pasteurized milk. The acidity data obtained from this series are portrayed in figure 1. The flavor results are shown in table 1.

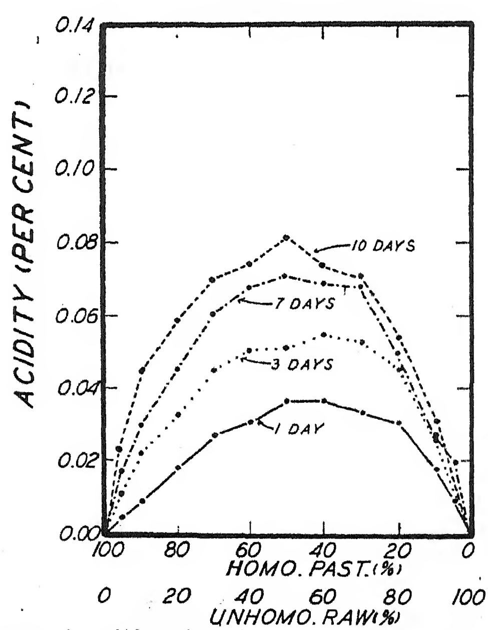

FIG. 1. The increase in acidity after different storage periods when unhomogenized raw milk was added to homogenized pasteurized milk in different proportions.

After three to five days storage, a slight increase in acidity over the control lot was noted in the homogenized pasteurized milk which contained as

little as one per cent of unhomogenized raw milk. When 5 per cent of unhomogenized-raw milk was added to the homogenized pasteurized milk an increase in acidity was observed after one day of storage. As the percentage of unhomogenized raw milk in the homogenized pasteurized milk was increased up to 50 per cent, a progressive increase in the titratable acidity occurred. The maximum increase in acidity was encountered when the ratio of unhomogenized raw milk to homogenized pasteurized milk was approximately one to one. Beyond this point, increased increments of unhomogenized raw milk resulted in a progressive decrease in acidity from the maximum. Small quantities of homogenized pasteurized milk in unhomogenized raw milk, such as one and three per cent, were sufficient to produce an increase in acidity after one to three days of storage, but seemed only slightly more effective in producing an increase in acidity than similar quantities of unhomogenized raw milk in the homogenized pasteurized milk.

TABLE 1

Development of rancidity due to lipolysis in milk made by mixing unhomogenized raw milk with homogenized pasteurized milk. (Average of three trials)

Sample		Rancidity* after			
% Unhomo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.33	0.67
5	95	0.00	0.33	1.00	2.67
10	90	0.33	1.00	2.33	2.67
20	80	2.00	2.67	3.33	3.67
30	70	2.00	3.00	3.33	3.67
40	60	2.33	4.00	4.00	4.00
50	50	2.67	4.00	4.00	4.00
60	40	2.67	4.00	4.00	4.00
70	30	2.33	4.00	4.00	4.00
80	20	2.67	3.00	3.33	3.67
90	10	2.00	2.00	3.00	3.33
95	5	0.67	0.33	1.33	3.00
99	1	0.00	0.00	0.33	0.33
100	0	0.00	0.00	0.00	0.00

* Flavor intensity designated by numerical values ranging from 0 (no rancidity) to 4 (pronounced rancidity).

A questionable rancid flavor was detected in some samples of homogenized pasteurized milk containing one per cent of unhomogenized raw milk after 7 to 10 days of storage, whereas the flavor was pronounced in those samples which contained five per cent of unhomogenized raw milk after the same storage period. A further increase in the percentage of unhomogenized raw milk added to the homogenized pasteurizd milk caused a more intense rancid flavor to develop and also produced the flavor more rapidly. All the samples containing from 10 to 90 per cent of unhomogenized raw milk developed a

pronounced rancid flavor upon storage, with the flavor being definite after one day of storage in mixtures of 20 per cent or more. When the sample contained less than 10 per cent of homogenized pasteurized milk in unhomogenized raw milk the speed and intensity of the rancid flavor development was decreased.

Lipolytic activity in mixture of homogenized raw and homogenized pasteurized milk. The acidity data obtained from mixtures of homogenized raw and homogenized pasteurized milk are presented graphically in figure 2

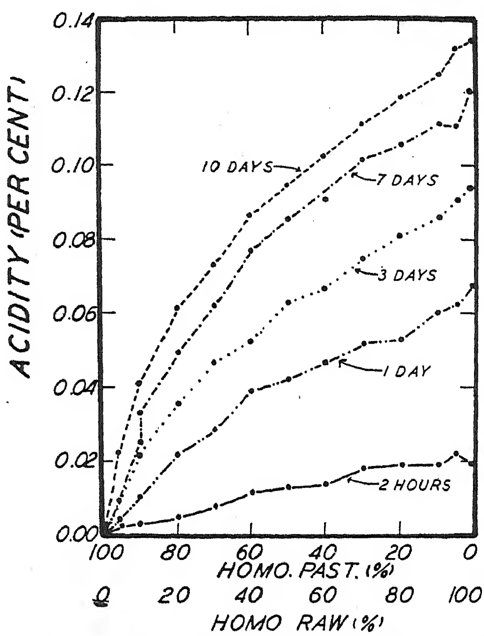

FIG. 2. The increase in acidity after different storage periods when homogenized raw milk was added to homogenized pasteurized milk in different proportions.

and the flavor results are presented in table 2.

When the homogenized raw milk was added to homogenized pasteurized milk, the acidity developed progressively as the increments of homogenized raw milk were increased. In the previous experiment the maximum acidity development occurred when the unhomogenized raw milk and homogenized pasteurized milk were mixed at a ratio of 1:1 In this experiment the maximum acidity developed in the 100 per cent homogenized raw milk. The slightly accelerated and persistent rate of lipolysis with such mixtures might be expected inasmuch as the maximum fat globule surface areas produced by the condition of the experiment were present throughout the series since both lots were homogenized. The development of rancid flavors, in general, closely followed the changes in titratable acidity.

TABLE 2

Development of rancidity due to lipolysis in milk made by mixing homogenized raw milk with homogenized pasteurized milk. (Average of three trials)

Sample		Rancidity after			
% Homo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.00	0.67
5	95	0.00	1.33	1.66	2.33
10	90	0.00	1.66	3.00	3.33
20	80	1.33	2.67	3.67	4.00
30	70	1.33	3.00	4.00	4.00
40	60	2.00	3.67	4.00	4.00
50	50	2.67	3.67	4.00	4.00
60	40	3.33	3.67	4.00	4.00
70	30	3.33	3.67	4.00	4.00
80	20	3.67	4.00	4.00	4.00
90	10	3.67	4.00	4.00	4.00
95	5	3.67	4.00	4.00	4.00
99	1	4.00	4.00	4.00	4.00
100	0	4.00	4.00	4.00	4.00

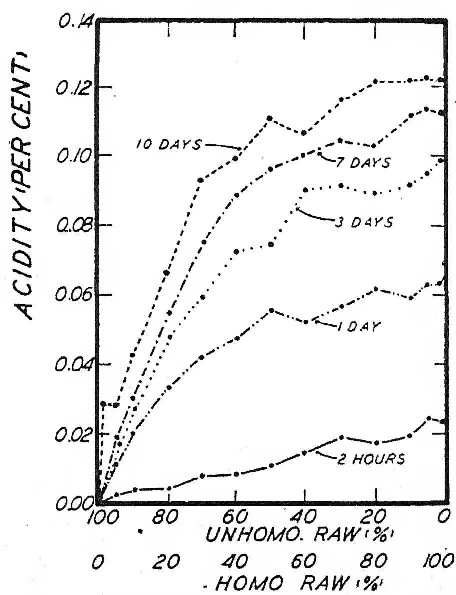

FIG. 3. The increases in acidity after different storage periods when homogenized raw milk was added to raw milk in different proportions.

Lipolytic activity in mixtures of unhomogenized and homogenized raw milk. The results secured when unhomogenized and homogenized raw milk were mixed in various proportions are shown by figure 3 and in table 3.

The same general trend in the development of rancidity and increased

acidity was noted in this series as with the second series. There seemed to be one exception, however; the acidity developed at a slightly faster rate as the percentage of homogenized raw milk in the unhomogenized raw milk increased up to 50 per cent, beyond which the increase was neither so rapid nor so great.

TABLE 3

Development of rancidity due to lipolysis in milk made by mixing raw milk with homogenized raw milk. (Average of three trials)

Sample		Rancidity after			
% Homo. raw	% Homo. pasteurized	1 day	3 days	7 days	10 days
0	100	0.00	0.00	0.00	0.00
1	99	0.00	0.00	0.00	0.00
5	95	0.00	1.00	1.00	2.00
10	90	1.50	2.00	2.00	2.50
20	80	2.00	2.50	3.00	3.50
30	70	2.00	3.50	3.50	3.50
40	60	2.00	4.00	4.00	4.00
50	50	2.00	4.00	4.00	4.00
60	40	2.00	4.00	4.00	4.00
70	30	4.00	4.00	4.00	4.00
80	20	4.00	4.00	4.00	4.00
90	10	4.00	4.00	4.00	4.00
95	5	4.00	4.00	4.00	4.00
99	1	4.00	4.00	4.00	4.00
100	0	4.00	4.00	4.00	4.00

Likewise, a slightly more intense rancid flavor was noted at one and three days in mixtures of homogenized raw with raw milk than similar mixtures with homogenized pasteurized milk. However, at the 10-day storage period, little difference was noted in the intensities of rancid flavor at comparable concentrations of homogenized raw milk.

DISCUSSION AND SUMMARY

A rancid flavor and an increase in acidity were found to develop readily on storage when raw milk was mixed with homogenized pasteurized milk. The results secured confirm earlier work (2, 3), that lipolytic activity is not confined solely to homogenized raw milk but to homogenized pasteurized milk as well provided active lipase is present. The maximum increase in acidity occurred when the ratio of raw milk to homogenized pasteurized milk was approximately one to one. As the percentage of raw milk in the homogenized pasteurized milk increased above 50 per cent, the increase in titratable acidity was found to be correspondingly less. When only a small percentage of the sample was homogenized pasteurized milk, very small increases in acidity occurred. These increases in titratable acidity were closely associated with the development of a rancid flavor.

The fact that the greatest increases in acidity in mixtures involving pasteurized milk occurred when the milk was approximately 50 per cent unhomogenized raw and 50 per cent homogenized pasteurized indicates that the amount of increased surface or increased surface activation caused by homogenization and the amount of lipase added by the raw milk are of approximately equal importance in the development of rancidity in homogenized milk. It would appear, therefore, that increases in acidity and the development of rancidity in homogenized raw milk are dependent upon the factors concerned with the increased surface and not upon an activation of lipase by homogenization.

Further evidence of the equal importance of the fat surfaces and the amount of lipase present is shown by the fact that when homogenized raw milk was added to homogenized pasteurized milk the rate of increase in acidity was only slightly greater than when unhomogenized raw milk was mixed with homogenized pasteurized milk. If lipase is activated by homogenization it would seem that these increases should have been considerably faster than those noted. The greater increase which did occur in the homogenized raw and homogenized pasteurized milk mixtures might be explained by the fact that all of the fat had been subjected to homogenization so that there was more fat surface exposed upon which the lipase could act than in the raw milk and homogenized pasteurized milk mixtures in which only a portion of the fat had been subjected to homogenization. In the latter case the amount of lipase added by the raw milk seemed to be the limiting factor in the development of rancidity. The lipase added to the homogenized pasteurized milk in the form of unhomogenized raw milk appeared to be just as effective in causing rancidity as was the lipase added by the homogenized raw milk.

From these studies it would seem that homogenized pasteurized milk contaminated with raw milk is as susceptible to lipolysis as homogenized raw milk. In addition, these results indicate the possibility if controlling the extent of the development of rancidity through the use of proper mixtures of homogenized pasteurized and raw milk.

CONCLUSIONS

Rancidity developed readily in mixtures of milk composed of (a) unhomogenized raw milk and homogenized pasteurized milk, (b) homogenized raw milk and homogenized pasteurized milk, and (c) unhomogenized raw milk and homogenized raw milk.

The development of rancidity seemed to be equally dependent upon the amount of lipase present and upon the amount of acceleration afforded by the newly created surfaces.

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EFFECT OF CERTAIN FACTORS UPON LIPOLYSIS IN HOMOGENIZED RAW MILK AND CREAM*

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The acceleration of lipase action in raw milk by homogenization is now generally accepted. This acceleration has been attributed by some to increased surface area afforded the lipase by the breakdown of the fat globules and by others to a re-surfacing of the fat globules by material more susceptible to lipolytic action. Irrespective of the actual cause for the enormous and rapid rate of lipolysis in homogenized milk, evidence is accumulating which indicates that factors which affect lipase action in normal milk may not have the same effect on lipase activity in the homogenized product. A limited amount of information illustrating these differences has already been published. Additional evidence is presented in this paper.

Lipolytic action on fat in homogenized milk has been previously studied (3, 4, 7). Gould and Trout (4) found the acid degree of the fat (expressed in milliliters of N/1 NaOH per 100 grams of fat) to increase four-fold to six-fold within a few minutes after homogenization, and to increase on an average of 1,652 per cent within 24 hours. The author (3) observed considerable lipolysis to have occurred in fat obtained from milk homogenized at temperatures of 105 to 135° F., whereas slight fat splitting occurred in milk homogenized at 145° F. These temperatures are considerably above those which have been found to be effective in greatly inhibiting lipase action in normal unhomogenized milk (9, 10, 11, 12).

Lipase action in homogenized milk is apparently not affected by temperature activation which brings about marked changes in normal milk (6, 7). Krukovsky and Sharp (7) believe the difference is due to the fact that in the homogenized product the "lipase is already in the active state as a result of the resurfacing of the milk fat. . . ."

Another point of difference between lipase activity of normal and homogenized milk pertains to the temperature coefficient. Krukovsky and Sharp (7) found the temperature coefficient of the lipase action to differ depending upon whether the fat globules were normal or whether they had been "resurfaced." Fat globules with natural surfaces showed more rapid lipolysis with lower temperature whereas a reverse condition occurred with the emulsified fat.

A relationship between oxidative changes in the fat and lipolysis in normal milk is indicated by Davies (1) and Krukovsky and Sharp (8). Davies found peroxide formation to occur simultaneously with lipase action; the

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peroxides being formed from oxidation of oleic acid which was freed by the fat splitting enzyme. This worker also found copper to be effective in inhibiting lipase activity, with 2 p.p.m. reducing lipolysis by approximately 70 per cent. Herrington and Krukovsky (5) found lipase action was reduced about 20 per cent by 0.2 and 0.4 p.p.m. of copper. Later, Krukovsky and Sharp (8) showed that the inactivating effect of copper, even in amounts of 2 to 8 p.p.m., was almost entirely prevented by removing the dissolved oxygen in the milk. They further found that the removal of oxygen increased the resistance of normal milk lipase to inactivation by heat. In earlier work, Dorner and Widmer (2) were unable to prevent rancidity in homogenized milk by removal of oxygen. However, rancidity was prevented by addition of carbon dioxide, but this was thought to be due to increases in the acidity of the milk by the gas.

In the study herein reported, results are presented which deal with the lipolysis which occurs in homogenized raw milk or cream and with the influence of certain factors upon the rate and extent of the lipolytic action.

EXPERIMENTAL PROCEDURE

Milk used in these trials was mixed-herd milk secured from the College creamery. Homogenization was at 500–1000 pounds pressure by means of a stainless-steel, commercial-size viscolizer. Milk or cream was homogenized at approximately 100° F., and a similar temperature was used when the raw milk was separated. To stop lipolysis following processing and storage, the milk or cream was pasteurized at 148–150° F. for 30 minutes. When a storage period was involved, a temperature of approximately 40° F. was used unless otherwise specified.

All measurements to determine lipolysis were conducted on the fat. Fat for analysis was obtained by churning the cream, followed by centrifuging and filtering the melted butter oil. The free fatty acids were measured by direct titration with 0.1 N NaOH using the procedure described previously (4). The values are expressed as acid degrees (the number of milliliters of 1.0 N NaOH per 100 grams of fat).

Although churning in many cases was comparatively difficult due both to homogenization and also to the subsequent lipolysis which frequently occurred, nevertheless, it was always possible to secure sufficient fat for the determinations. Perhaps the greatest churning difficulty was encountered with those samples containing relatively large quantities of formalin, this effect doubtless being partly due to the action of formalin on the proteins.

Peroxide values were determined by the Wheeler method (13), and the results are expressed as peroxide number (the millimols of peroxide oxygen in combination with one kilogram of fat).

EXPERIMENTAL RESULTS

Influence of copper on lipolysis. In this experiment, copper was added as a solution of copper sulfate to make concentrations in the milk of 2, 6 and 10 p.p.m. The copper was added to the milk before homogenization in certain trials and following homogenization in others. The results of several trials are shown in table 1.

TABLE 1

*Lipolysis in fat from homogenized milk as influenced by added copper**

Sample	Hours	Trial No. 1	2	3	4	Ave.
Control	0	3.67	4.55	3.40	4.20	3.96
	24	11.70	18.10	14.55	14.30	14.66
	72	14.30	23.70	15.20	19.60	18.20
2 p.p.m. Cu	24	12.15	18.80	15.95	13.20	15.03
	72	14.40	25.50	17.30	16.80	18.50
6 p.p.m. Cu	24	11.75	16.95	13.15	11.15	13.25
	72	15.00	24.60	15.20	14.50	17.20
10 p.p.m. Cu	24	11.75	18.80	12.60	13.25	14.10
	72	14.70	24.60	14.50	16.10	17.48

* Values expressed as acid degrees. Copper added following homogenization in first two trials and before homogenization in last two trials.

These results show the copper to have no significant effect on the extent of lipolysis whether added prior to, or subsequent to, homogenization. The acidity values for the copper-containing samples were practically the same as the control samples in every trial after 24 and 72 hours. Average values of the four trials show no distinct trend in fat acidity to accompanying increases in the copper content. The failure of copper, even in comparatively large amounts, to inhibit lipolysis in homogenized milk is at variance with the results reported by others for lipase action in the unprocessed product.

Influence of sodium chloride on lipolysis. Information is lacking concerning the influence of NaCl on lipolysis in homogenized milk or cream, although Pfeffer, *et al.* (10) report that NaCl was found to inhibit lipolysis in the unhomogenized product. Because of the scarcity of information on this subject, trials were conducted in which different concentrations of NaCl were added to homogenized raw cream In these trials, NaCl was added to the cream, at the rate of 0, 2, 5, and 8 per cent and the cream stored for 72 hours. The results are illustrated by figure 1.

This figure shows NaCl to have an inhibiting effect upon fat splitting, with the effect increasing directly with the salt concentration. The broken line represents the acid degree of the fat at the time of adding the salt. The results show that both the 5 and the 8 per cent levels were sufficient to inhibit lipolysis practically completely. If the 8 per cent concentration is taken

to be 100 per cent efficient in preventing lipase activity, then the calculated efficiencies of the 2 and 5 per cent levels would be 41 and 94 per cent respectively. On the basis of these findings it would appear that the lipase activity in homogenized milk or cream is retarded and even prevented by NaCl.

Influence of formalin on lipolysis. The recent work of Herrington and Krukovsky (5) dealing with the use of formalin in unhomogenized normal milk indicates that even small quantities of this chemical reduced the lipase action to a small fraction of its original value and that larger amounts were

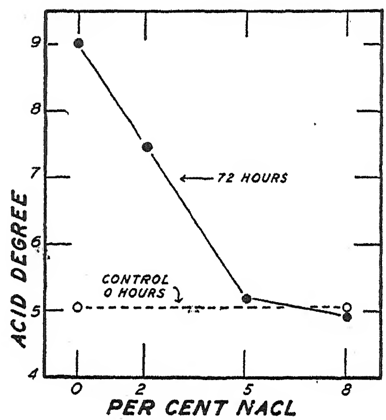

FIG. 1. The influence of sodium chloride on lipolysis in homogenized raw cream.

no more effective. Since the influence of formalin on lipolysis in homogenized milk or cream has not been studied, an experiment was conducted in this connection. Milk was warmed to 100° F., homogenized, and separated. The cream was standardized to 15 per cent fat and divided into 6 lots. These lots were treated as follows: Lot 1—Control—stored 0 hour; Lot 2—Control—stored 72 hours; Lot 3—5 ml. formalin per 3 pounds cream, stored 0 hour; Lot 4—1 ml. formalin per 3 pounds cream, stored 72 hours; Lot 5—3 ml. formalin per 3 pounds cream, stored 72 hours; Lot 6—5 ml. formalin per 3 pounds cream, stored 72 hours. The ratios of the formalin to cream were about 1:1350, 1:450, and 1:250. The results are presented in table 2.

These results show formalin in the amounts used to have no inhibitive effect upon the fat splitting action. The average values show the control lot to have changed from an acid degree of 4.97 to 8.25 within the 72 hour period, whereas the lot containing 1 ml. formalin underwent approximately the same extent of change and those with 3 and 5 mls. of formalin averaged even greater fat splitting during storage. A higher degree of lipolysis in the samples containing 3 and 5 mls. of formalin resulted in two of the three trials conducted.

TABLE 2

*Lipolysis in fat from homogenized milk as influenced by formalin**

Trial No.	Storage period (hours)					
	0		72			
	Formalin (ml.)		Formalin (ml.)			
	0	5	0	1	3	5
1	5.15	4.85	8.25	7.95	11.25	11.40
2	4.05	4.75	8.00	7.75	8.30	7.65
3	5.70	5.60	8.50	8.50	10.80	10.15
Avg.	4.97	5.07	8.25	8.07	10.12	9.73

* Formalin concentration as milliliters per 3 pounds of cream. Values expressed as acid degrees.

Influence of storage temperature on lipolysis. Dorner and Widmer (2), by using direct titration methods on homogenized milk, came to the conclusion that lipolytic activity varied directly with the storage temperature. Since the direct titration on the milk is a less sensitive method of measuring the acidity as produced by lipase activity, it appeared desirable to study the influence of storage temperature by means of fat titration. Therefore, an experiment was conducted in which 20 per cent cream was homogenized and then divided into four lots. Lot 1 was pasteurized at once; Lot 2 was stored for 72 hours at approximately 0° F., Lot 3 was stored for 72 hours at 35° F., and Lot 4 was stored for 72 hours at approximately 70° F. All of the samples were treated with a small amount of formalin (2 ml. per gallon), immediately following pasteurization to prevent excessive bacterial changes during storage. The results are illustrated by figure 2.

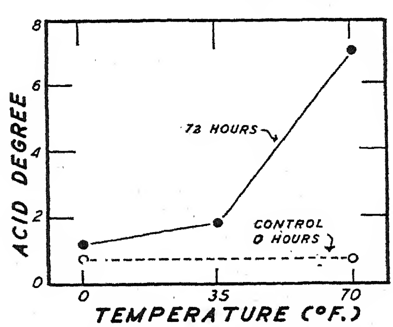

FIG. 2. The influence of the storage temperatures on lipolysis in homogenized raw cream.

These results show the lipase activity in homogenized cream to vary directly with the storage temperature. However, the average increases in free fatty acids at the two lower temperatures were slight, amounting to approximately 0.5 and 1.15 acid degrees for the 0° F. and the 35° F. tem-

peratures, respectively. Much greater lipase activity occurred at 70° F., with the acid degree increasing approximately 6.25 during the 72-hour period. Thus, the lipolytic activity was practically doubled between 0° F. and 35° F., and had increased approximately 12 fold at 70° F. These results had been secured prior to the appearance of the paper by Krukovsky and Sharp (7) dealing with "resurfaced" fat globules, but the same conclusions may be drawn even though the results were secured by somewhat different means, *i.e.*, that lipolysis in homogenized milk displays a normal temperature coefficient.

Influence of pasteurization of different milk fractions on lipolysis. Dorner and Widmer (2) found that when heated homogenized milk was mixed with raw skim milk the product became rancid. They concluded therefore that the causative agent was in the milk serum. Pfeffer, Weckel and Jackson (10) report similar conclusions for unhomogenized milk. In both of these studies the workers were actually referring to milk plasma rather than to milk serum. To study the problem of homogenized milk from the standpoint of changes in the acidity of the fat, trials were conducted in which fractions of 40 per cent cream and skim milk were remixed to make milk testing approximately 6 per cent. This prepared milk was then homogenized. In one lot the cream was pasteurized prior to mixing with the skim milk, in another lot the skim milk was pasteurized, whereas a third lot (the control) consisted of a mixture of the raw cream and raw skim milk. Fat acidity determinations were made at 0, 24, and 72 hours. The results are shown by figure 3.

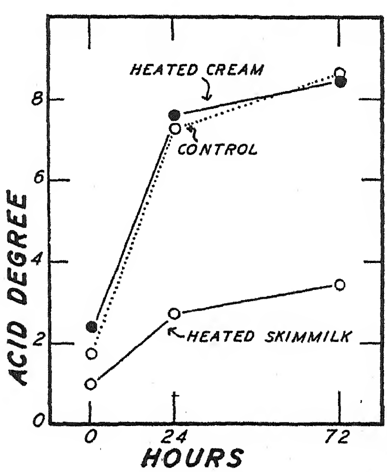

FIG. 3. The influence of heating cream and skimmilk fractions on lipolysis accelerated by homogenization.

This figure shows that although the heating of the skim milk markedly reduced the extent of lipolysis, a similar treatment of the cream had but

slight influence. It may be observed that the rate and amount of lipolytic action was not greatly different between the control lot and the lot in which the cream portion was heated. The fat acidity in the lot in which the cream was heated increased approximately 3.5 fold and the fat acidity in the control samples increased approximately 4.9 fold, thus indicating a somewhat greater increase in lipolysis in the latter. However, the actual percentage increase on the basis of the value at the 0-hour period may be of only secondary importance. For example, the change in fat acidity in the lot containing the heated skim milk amounted to approximately a 3.3 fold increase. Since the value at the 0-hour period was comparatively low, being approximately one-half that of the other lots at the same period, the total increase was much smaller than in the other lots. The increase during the 72 hour period in acid degrees amounted to 2.43 for the heated skim milk lot, as contrasted with 6.1 and 6.85 for the heated cream and control lots, respectively. The lipolysis which resulted when the skim milk fraction was pasteurized is doubtless due to the lipolytic activity of the plasma portion of the raw cream.

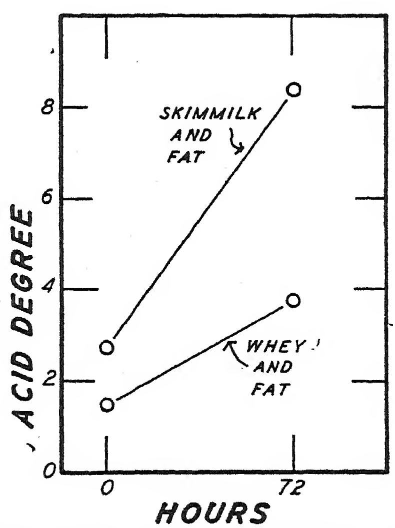

Fig. 4. Differences in lipolysis when fat is dispersed in skim milk and whey.

Influence of fractions of milk plasma on lipolysis. In this experiment, efforts were expended to determine the difference between skim milk and whey in affecting "homogenization" lipolysis. Raw skim milk was divided into two lots. In one lot the casein was precipitated by rennin and the whey filtered free of the coagulated protein. Melted butter oil secured from pasteurized cream was added both to the skim milk and the whey fractions to make a product testing approximately 4 per cent fat. The two lots were then homogenized, and fat was obtained for titration at 0 and 72 hours of storage. The results are portrayed by figure 4.

This figure shows greater lipolysis to occur in the case of skim milk-fat combination, although the whey-fat lot exhibited considerable lipolytic activity. The acid degree of the fat in the whey lot increased from 1.49 to 3.87, an increase of approximately 2.6 fold, whereas the acid degree change in the fat of the skim milk lot was from 2.69 to 8.44, an increase of about 3.1 fold. The fact that the whey was found to exhibit considerable lipolytic activity is at variance with the suggestion of Dorner and Widmer (2) to the effect that whey probably does not contain the agent which caused homogenized raw milk to become rancid. The results of this study do indicate, however, that a considerable portion of the lipolytic activity of milk is removed with the casein.

Relationship of lipolysis to oxidative changes in the fat. The results of Davies (1) and Krukovsky and Sharp (8) with lipase in normal milk indicate that oxidative changes are also involved with the lipolytic action. However, these workers found normal milk lipase to be inhibited by copper, whereas the results presented earlier in this paper show copper to be ineffective in preventing lipolysis in homogenized milk. This indicates that there may be no relationship between oxidative changes and lipolysis in the case of homogenized raw milk.

To determine if oxidative changes were occurring simultaneously with lipolysis, peroxide determinations* were conducted on the fat in the majority of the trials which were made in connection with this study. A summary of the results is given in table 3.

TABLE 3

Peroxide numbers of fat which has undergone different degrees of lipolysis

Number of samples	Acid degrees	Peroxide numbers	
		Average	Range
19	4 or less	0.32	0.00–0.65
4	4.1–6.0	0.31	0.14–0.50
19	6.1–8.0	0.39	0.01–1.00
5	8.1–10.0	0.32	0.15–0.60
35	10.1 or more	0.24	0.04–0.85

The results in this table offer evidence that lipolytic activity, accelerated by homogenization, is not related to oxidative changes, at least under the conditions of this experiment. The peroxide values were not significantly altered by marked changes in the degree of fat splitting and all of the values were relatively low indicating no appreciable amount of fat oxidation. On the basis of these results, it would appear that lipolysis in homogenized raw milk proceeds independently of oxidative changes in the fat.

* Credit is due Mr. R. C. Townley, graduate assistant in Dairy Manufactures, for the peroxide determinations.

DISCUSSION AND SUMMARY

The results secured in this study show that lipase action which occurs in homogenized raw milk usually reacts to external factors differently than does the lipase in normal milk. Other workers (1, 5, 8) have shown normal lipase activity to be greatly inhibited by copper, whereas in the studies herein presented, no such influence was detected in homogenized raw milk. Further, the work of Davies (1), and Krukovsky and Sharp (8) indicates oxidative changes occur simultaneously with, or perhaps precede, normal lipase action, but in these studies on "homogenization" lipolysis, no oxidative changes could be detected by means of the peroxide determinations, even though large amounts of fat splitting had occurred.

The fact that formalin had no inhibiting effect on lipolysis in homogenized raw milk would indicate that the lipase action in this product is different from that of normal milk, since Herrington and Krukovsky (5) found that formalin markedly lowers the lipase action in normal milk. However, Tarassuk (12) reported a study of milk from one cow in which formalin did not influence the activity of the lipase. On the basis of their formalin studies, Herrington and Krukovsky (5) expressed the belief that there are two lipases in milk, one of which is not affected by formalin. Dorner and Widmer (2) had previously suggested the presence of two lipases, one of which is extremely heat labile and which produces a sharp, bitter taste and marked acidity changes. These findings may indicate that the lipase in homogenized milk is different from the one responsible for the major portion of lipolytic activity in normal milk. However, additional proof of this is needed before definite conclusions may be drawn.

Further results of this study show that increasing the NaCl content decreases the lipolysis, whereas the lipase activity is increased by increases in the storage temperatures. Heating of the cream and skim milk fractions indicates that the lipase agent follows the plasma phase. Further, the lipolysis in a prepared fat-skim milk product was greater than in a similarly prepared mixture of fat and whey, although the fat dispersed in the whey did undergo appreciable splitting.

CONCLUSIONS

Lipolysis in homogenized raw milk is not affected, in all cases, by the same factors which have been found to influence the rate of fat splitting in normal milk. Whether these variations are due to different lipases or whether merely due to physical or physico-chemical changes involving the fat globules has yet to be definitely determined.

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OXIDATION-REDUCTION POTENTIALS AND THE OXIDIZED FLAVOR IN HOMOGENIZED MILK*

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Various workers have observed that homogenization tends to stabilize milk against oxidative changes (2, 3, 7, 11, 12, 13), but the mechanism by which the stabilization is produced has not been definitely ascertained. Ross (7), Dahle (1), and Thurston (11) expressed the belief that the adsorbed layer around the fat globules is the protective agent involved in homogenization. Earlier, Tracy, Ramsey and Ruehe (12) indicated that certain physical changes in the milk were involved which might have made the oxidized flavor less detectable.

Efforts have been made to correlate oxidation-reduction potentials with oxidized-flavor development. However, the results secured by Thurston (9), Greenbank (6), Webb and Hileman (14), and Fox (4) indicate that the potential values were not a definite indication of the tendency of normal milk to become oxidized. More of a relationship between oxidation-reduction potentials and oxidized-flavor development would be expected when the oxidation is induced by copper since Tracy, Ramsey and Ruehe (12), Gebhardt and Sommer (5), Thurston (9), Webb and Hileman (14), and Swanson and Sommer (8), found copper to cause a rise in the potential.

Although the role of homogenization in the control of flavor has been previously studied, no consideration has been given to the oxidation-reduction changes which may occur in homogenized milk under different conditions. Consequently, this study was conducted with the view of ascertaining these changes.

EXPERIMENTAL PROCEDURE

Mixed-herd milk obtained from the College creamery was used in the major portion of these studies. Pasteurization was accomplished at 143–145° F. for 30 minutes in stainless steel equipment. The milk was homogenized at the pasteurization temperature and at 2500 pounds pressure with a new style commercial-size viscolizer. The milk was cooled at once and stored at 34–40° F.

When copper was used, it was added as a weak solution of copper sulfate following the pasteurization and homogenization of the milk.

Oxidation-reduction potentials were determined by means of a Beckman pH meter using a bright platinum wire electrode in circuit with a saturated calomel cell. Usually about fifteen to twenty minutes were required before constant results could be obtained.

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Organoleptic examinations were made by at least two experienced milk judges. The samples were numbered in such a manner that their identity remained unknown to the judges. The intensity of the oxidized flavor was indicated as follows: 0—no flavor; 1—questionable; 2—slight; 3—distinct; 4—strong.

EXPERIMENTAL RESULTS

Preliminary studies. A large number of preliminary trials were conducted in which milk was utilized which was normally susceptible to oxidized-flavor development. This milk was secured direct from the College farm. The results of these preliminary experiments showed the unhomogenized milk to become oxidized on storage, whereas the homogenized milk did not develop this defect. However, there was no significant difference in the oxidation-reduction (Eh) of the unhomogenized and homogenized milk, both of these milks tending to increase in potential during storage. These preliminary trials were the basis for additional studies.

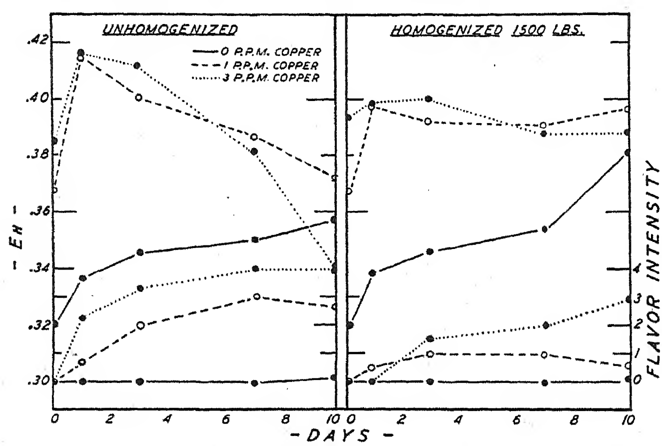

FIG. 1. Changes in oxidation-reduction potentials and flavor in unhomogenized and homogenized milk containing added copper. (Homogenization pressure 1500 pounds. Lower three curves represent flavor intensity.)

Oxidation-reduction potentials and oxidized flavor in copper-treated homogenized and unhomogenized milk. In these experiments trials were conducted in which the oxidized flavor was induced by the addition of copper to milk which was normally not susceptible to oxidation. Copper was added in concentrations of 0, 1, and 3 p.p.m. to homogenized and unhomogenized milk. Homogenization pressures used were 1500 and 2500 pounds.

The results of the trials in which the milk was homogenized at 1500 pounds pressure are shown in figure 1. Results are shown both for the unhomogenized and for the homogenized milk.

This figure shows the close similarity between the Eh values of the

unhomogenized and homogenized milk, irrespective of the differences in flavor changes. The lots which contained no added copper show a gradual change upward during the ten-day period. This occurred both in the homogenized and unhomogenized product. The addition of copper, either 1 p.p.m. or 3 p.p.m., markedly increased the potential of the milk. The most abrupt Eh rise in the copper-contaminated samples occurred during the first day; thereafter, the potential tended to decrease, with the decrease being especially noticeable in the unhomogenized milk.

From the standpoint of flavor, the unhomogenized milk to which copper was added developed distinct to strong oxidized flavors before the third day, with the 3 p.p.m. samples becoming oxidized within 24 hours. The untreated-unhomogenized milk remained practically free of oxidized flavor during this 10-day period. The untreated-homogenized milk, likewise, was free from oxidized flavor during the storage period, and the milk containing 1 p.p.m. of added copper showed only an extremely slight tendency towards oxidation. However, the 3 p.p.m. of copper were sufficient to overcome the stabilizing ability of 1500 pounds of homogenization as shown by the fact that the samples containing this amount of copper gradually developed a higher intensity of the oxidized flavor during storage. The oxidized flavor in the homogenized milk developed more slowly and to a lesser extent than in the unhomogenized milk similarly treated with copper.

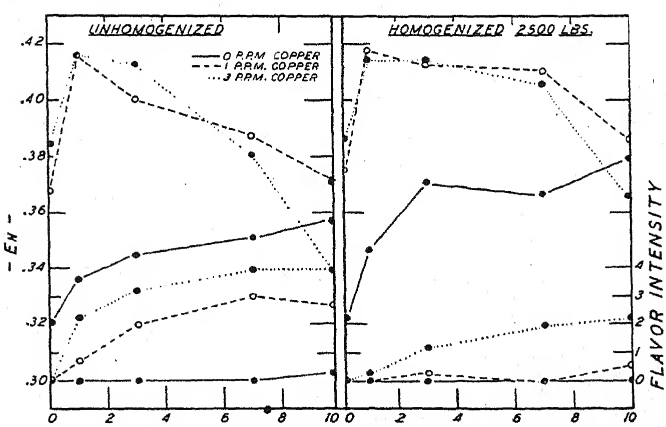

FIG. 2. Changes in oxidation-reduction potentials and flavor in unhomogenized and homogenized milk containing added copper. (Homogenization pressure 2500 pounds. Lower three curves represent flavor intensity.)

The results secured when 2500 pounds of pressure were used are illustrated by figure 2. These results are not greatly different from those in which 1500 pounds pressure was used. Again, the Eh values for the homogenized milk were similar to those for the unhomogenized, with the

copper causing marked increases in the potential in both cases. Flavor changes were also similar between these trials and those shown in figure 1. The 2500 pounds pressure was sufficient to prevent the development of the oxidized flavor in the presence of 1 p.p.m. of added copper but was unable to protect entirely the milk when 3 p.p.m. of copper were used. It should be emphasized in this connection that the copper was added following homogenization; thus the flavor stabilizing ability of the homogenization process is less than if the copper had been added prior to the pressure treatment (7, 13).

In general, the results illustrated in figures 1 and 2 show that oxidation-reduction potential changes are not definitely related to copper-induced oxidized flavor when homogenization is involved. The addition of copper does increase the potential in homogenized milk, whereas the oxidized flavor may or may not develop, depending on the protective power of the homogenization process.

SUMMARY AND CONCLUSIONS

Homogenization of milk tends to stabilize the milk against oxidation but has no influence on changes in the oxidation-reduction potentials. Trends in Eh were similar regardless of homogenization.

The mechanism by which homogenization prevents or retards oxidized-flavor development would appear not to be associated with oxidation-reduction potentials.

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LIVE WEIGHT OF COW AT VARIOUS STAGES OF LACTATION IN RELATION TO MILK-ENERGY YIELD

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In connection with the postulate previously advanced (1) that milk-energy yield per unit live weight is independent of live weight, it seems desirable to emphasize that the postulate is based on live weight at the start of the lactation and milk-energy yield for the first 8 months of the lactation. The 8-months feature avoids complications of advanced pregnancy, and probably affords as good a biological measure of dairy development as any longer period of the same lactation, even in farrow cows.

What is the relation of live weight to the 8-months yield, and how is it affected by the stage of lactation at which live weight is measured? In the present paper some published data by Dawson, Kopland and Graves (2)[1] are utilized in answer to the above question, particularly with reference to the effect of stage of lactation at which live weight is measured.

ANALYTICAL PROCEDURE AND RESULTS

Another way of expressing the above postulate is to say that yield is proportional to live weight, and the conformity of observed data to the postulate may be tested by fitting the equation, $FCM = aW^b$, (FCM = milk-energy yield in pounds of 4 per cent milk per day for the partial lactation; W = live weight of cow in pounds). If the exponent b, turns out to be 1, it indicates FCM is proportional to W; if more than 1, large cows yield more per unit live weight than small ones; if less than 1, small cows yield more per unit live weight than large cows. Primary interest with the present data (2) however is to see how the exponent, b, is affected by stage of lactation at which live weight is measured. From the published data live weight is taken at the first month of lactation; the second month of lactation; and so on to and including the twelfth month of lactation, with finally live weight as an average of the 12 monthly weights.

The exponent, b, in the equation, $FCM = aW^b$, has been determined by fitting a straight line, $FCM = a' + b'W$ and approximating b as, $b = b'(\overline{W}/\overline{FCM})$, where the overscore indicates the mean.[2] The values of b thus derived are given in table 1.

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[1] The authors have generously supplied the monthly fat percentage data, supplementing their Table 11, which permit the computation of monthly milk-energy yields.

[2] On the theory that FCM/W is a fundamental criterion of dairy development, it is desirable to have FCM/W for individual cows and lactations. In case the individual FCM/W's have been fitted with the equation, $FCM/W = a'' + b''W$, (as in 3, fig. 1), b in the power equation may be approximated as $b = 1 + b''(\overline{W}/\overline{FCM/W})$. The derivation of these approximations will be explained in a later paper.

TABLE 1

*Relation of live weight in pounds (W) at various stages of lactation to partial lactation milk-energy yield (FCM)**

Month of lactation	Live weight, W			Correlation, r_{WFCM}		Value of b in $FCM = aW^b$†	
	Lowest	Highest	Average	10-mo.	8-mo.	10-mo.	8-mo.
1	1125	1399	1284	0.62	0.70	1.11	1.07
2	1140	1346	1236	0.64	0.67	1.47	1.32
3	1164	1374	1243	0.64	0.68	1.56	1.41
4	1172	1345	1250	0.66	0.67	1.70	1.49
5	1174	1334	1251	0.61	0.58	1.72	1.39
6	1133	1331	1250	0.52	0.54	1.21	1.07
7	1151	1342	1255	0.49	0.51	1.18	1.05
8	1156	1348	1261	0.25	0.25	0.71	0.60
9	1180	1369	1271	0.13	0.12	0.35	0.28
10	1207	1424	1290	0.32	0.34	0.69	0.63
11	1203	1469	1311	0.37	0.43	0.72	0.72
12	1261	1505	1343	0.30	0.38	0.60	0.64
Av.	1195	1342	1271	0.53	0.57	1.41	1.29

* This table is based on 11 records of 8 Holstein cows (2). The cows were fed exclusively on alfalfa hay throughout (consuming up to 48 pounds per cow per day). Because of deferred breeding the 10-months partial lactation FCM is included, in addition to the 8-months, since no cow carried a calf more than 169 days during the 10-months partial lactation. All cows were in the second or later lactations. The last line of the table deals with W as an average of the 12 monthly W's.

† The formula for deriving b, as given in the text, shows that in the power equation the exponent, b, is (approximately) the linear regression in terms of the means. The coefficient of correlation, r, is the linear regression in terms of the standard deviations. In the 8-months partial lactation, for example, the FCM series is identical for each of the 12 months, and any differences in the r/b ratios, as between months, must arise in differences in variability in live weight ($\sigma_W/\overline{W}$) as between months.

From the last column of table 1 it is observed that the exponent, b, in the power equation, is 1.07 where live weight is measured in the first month of lactation; reaches a high of 1.49 where live weight is measured in the fourth month of lactation; and reaches a low of .28 where live weight is measured in the ninth month of lactation. The relation of yield to live weight is greatly affected by the stage of lactation at which live weight is measured.

It will be noted that the coefficients of correlation between weight and yield follow a somewhat different cycle of changes than b. The highest correlation is found at the first month of lactation. On the whole it appears that if a single live weight determination is made at each lactation, the most desirable time is during the first month after calving. Furthermore, for practical use in the field (in distinction to experimental work), it seems that this one point of measurement in each lactation may be quite adequate.[3]

[3] The postulate that FCM/W is independent of W (8-months FCM and first-month W) is set up as a philosophy of what may reasonably be expected as between dairy cows of varying sizes, rather than as a statement of what now prevails among dairy cows. While the present 11 records are too few in number to be at all conclusive, statistically, they do afford an instance of observation in which FCM/W, within herd and within breed,

SUMMARY AND CONCLUSIONS

Eleven records of Holstein cows are fitted with the equation, $FCM = aW^b$, in which W is live weight measured successively at monthly intervals during the lactation period, and FCM is milk-energy yield for the first 8-months of the lactation period. There is a vast difference in the resulting value of b (.28 to 1.49), according to the stage of lactation at which live weight is measured. FCM and W are most closely related where live weight is measured in the first month of lactation ($r = .70$).

The above records and other experimental evidence indicate that, within a dairy breed and within a herd (comparable environment) FCM is proportional to the 1.07 power of live weight, where live weight is measured in the first month of lactation. Both practical and biological considerations indicate the desirability of estimating live weight of the cow in the first month of each lactation in D.H.I.A. and similar milk-recording work. Measuring live weight in this way, it appears sound to measure lactation performance of the cow at each lactation in terms of FCM/W, that is, milk-energy yield for the 8-months partial lactation per unit live weight in the first month of the lactation.

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is substantially independent of W, that is, referring to the observed fact that FCM is proportional to the 1.07 power of live weight.

Another similar instance is afforded by a previous analysis (3) of 66 records of Holstein cows (heavily grain fed) reported by the Cornell Station, including accurate initial live weight and partial lactation milk-energy yield for 280 or 259 days. From these records (3, fig. 1) applying the method of footnote 2, it is found that $b = 1.07$, as an average. That is, in these experimental records, also, FCM is proportional to the 1.07 power of live weight.

The only other records, based specifically on first-month weight and 8-months yield, known to the writer consist of a more numerous (1152) but less accurate body of D.H.I.A. records, in which it was found (4, footnote 3) that within herd and within breed (Holstein or Jersey) FCM was proportional to about the $\frac{2}{3}$ power of live weight. It now comes to light that the scale of the chest-girth live-weight tape used in estimating live weight was erroneous, grossly over-estimating the weight of large cows and grossly underestimating the weight of small cows. It appears likely that removal of this bias in the weight estimate would result in showing in these records also that FCM was proportional to the first-month live weight (rather than the $\frac{2}{3}$ power of weight).

THERMODURIC BACTERIA IN MILK. III. THE EFFECT OF CHANGING AGAR AND TEMPERATURE OF INCUBATION FOR PLATE COUNTS ON THE PROBLEM OF THERMODURIC BACTERIA IN MILK

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In 1939 the American Public Health Association (1) adopted a much richer medium than had hitherto been used as the standard for plate counts of bacteria in milk. It has also been proposed that the temperature of incubation for the plates be reduced from 37° C. to 32° C. (2). Several investigations of the effect of the new medium on counts of various dairy products have been published (3, 4, 5, 6, 7, 8). These investigations do not clearly show whether the per cent increase in count is different in the case of raw milk than it is in the case of pasteurized milk, although several investigations on a medium similar to the new standard medium (tryptone glucose skimmilk agar) indicate that pasteurized milk gives a much greater per cent increase in count than does raw (9, 10, 11). Similarly, the work of Kelly shows that lowering the temperature of incubation from 37° C. to 32° C. causes a greater per cent increase in count with pasteurized milk than with raw milk (12). These reports would seem to indicate that either enrichment of the agar or lowering of the temperature of incubation would tend to favor the growth of thermoduric bacteria. However, the comparisons available in the literature are not direct comparisons of counts on the same milk before and after pasteurization, and there is, in general, little known about the history of the samples. For that reason it seemed desirable to determine the effect of these variations in methods of enumerating bacteria on the counts on the same milk both before and after pasteurization, so that it would be possible to state clearly the effect of changing the method of counting on the number of thermoduric bacteria disclosed and on the per cent of the total organisms counted in the raw milk which are reported to be thermoduric.

EXPERIMENTAL

The work reported here was done in a bottling plant receiving approximately 70,000 pounds of milk daily from about 300 producers. One hundred lots of milk were examined. For each lot, about 300 gallons of raw milk were drawn into a glass-lined pasteurizer, and after agitation a sample was withdrawn in a sterile vial by dipping the vial (held in a clamp) into the milk. The milk in the pasteurizer was heated to 143°–144° F. (61.6°–62.2° C.), held for 30 minutes, and cooled in the pasteurizer to about 130°

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F. (54.4° C.). A sample of this pasteurized milk was withdrawn in a sterile vial, quickly cooled in ice water, and used for a bacteria count.

The raw milk samples were divided into three portions. One was used for a bacteria count while raw. A second was pasteurized in the laboratory at 143°–144° F. for 35 minutes. A third was pasteurized in the laboratory at 160.5°–161.5° F. (71.4°–71.9° C.) for 16 seconds. Heating to pasteurizing temperature required about 27 minutes in the plant pasteurization, 4 minutes in the laboratory low-temperature pasteurization, and 2.5 minutes in the laboratory high-temperature pasteurization. Cooling was very rapid in both laboratory pasteurizations, and cooling to 130° F. in the plant required about 5 minutes.

Four plates were made on each sample, or 1600 plates for the 100 lots of milk. Two of the four plates were poured with the old standard nutrient agar, and two with tryptone glucose extract milk agar, both media being made from Difco Dehydrated products. One plate with each agar was incubated at 37° C., the other at 32° C., the incubation period being very close to 48 hours. A Quebec Colony Counter was used in counting colonies. Adequate blanks were made to assure sterility of all materials.

The experiments covered a period from October, 1940, through January, 1941. Because of the large amount of space that would be required to show in detail each of the 1600 counts, averages were made of the counts on the

TABLE 1

Averages of bacteria counts per milliliter on 100 lots of milk when raw and after pasteurization by three methods, when determined on two agars and at two temperatures of incubation

Treatment of milk	Method of counting		Bacteria per milliliter	Per cent of count on old agar at 37° C.	Per cent of count on new agar at 32° C.	Per cent of raw milk count (or per cent of thermodurics)
	Designation	Description				
Raw	A	Old agar at 37° C.	72,830	100.0	57.4	....
	B	Old agar at 32° C.	101,230	138.9	79.8	....
	C	New agar at 37° C.	90,540	124.3	71.3	....
	D	New agar at 32° C.	126,820	174.1	100.0	....
Pasteurized in the plant at 61.6° C. for 30 minutes	A	Old agar at 37° C.	6,725	100.0	26.5	9.2
	B	Old agar at 32° C.	13,769	204.7	54.2	13.6
	C	New agar at 37° C.	15,045	223.7	59.2	16.6
	D	New agar at 32° C.	25,395	377.6	100.0	20.0
Pasteurized in the laboratory at 61.6° C. for 35 minutes	A	Old agar at 37° C.	4,806	100.0	21.9	6.5
	B	Old agar at 32° C.	11,577	240.8	52.7	11.4
	C	New agar at 37° C.	12,226	254.3	55.7	13.5
	D	New agar at 32° C.	21,932	456.3	100.0	17.2
Pasteurized in the laboratory at 71.6° C. for 16 seconds	A	Old agar at 37° C.	5,319	100.0	24.3	7.3
	B	Old agar at 32° C.	12,568	236.2	57.5	12.4
	C	New agar at 37° C.	12,512	235.2	57.2	13.8
	D	New agar at 32° C.	21,853	410.8	100.0	17.2

raw and the three kinds of pasteurized milk, as determined on the two agars and at the two temperatures of incubation. These average counts are shown in table 1 in the first or left-hand column.

The second column is designed to show the numerical relationship between the counts on the old agar at 37° C. and the counts by the other three methods of counting.

The third column is similar to the second, except that it shows the relationship between the count on the new agar at 32° C. and the counts by the other three methods of counting. If it is assumed that the count on the new agar at 32° C. gives the maximum possible count, or a count that approaches all of the organisms present (an assumption that is certainly not entirely justified), then the figures given in the third column represent the percentage of the total organisms present that will grow under the conditions specified.

The fourth or right-hand column shows the per cent of the organisms present in the raw milk, as determined by the four methods of counting, that will survive each of the three methods of pasteurization.

It is possible, on the basis of the counts made by the four methods, and designated in table 1 as A, B, C and D, to divide the organisms growing on the plates into four groups (shown in table 2 as classes 1, 2, 3 and 4), as follows:

1. Those that will grow on the old agar at 37° C. This is obviously the total count on the old agar at 37° C.
2. Those that require the temperature of incubation to be reduced from 37° C. to 32° C. but do not require enrichment of the agar. This is the difference between the count on the old agar at 32° C. and that on the old agar at 37° C. or (B–A).
3. Those that require enrichment of the agar but not reduction of the temperature of incubation. This is the difference between the count on the new agar at 37° C. and the count on the old agar at 37° C. or (C–A).
4. Those that require both enrichment of the agar and reduction of the temperature of incubation. This is a more complicated calculation, involving all four counts. It is done as follows:
    a. If A, the count on the old agar at 37° C., is subtracted from C, the count on the new agar at 37° C., the result is the effect on the count of changing the agar at 37° C. This may be expressed at (C–A).
    b. If B, the count on the old agar at 32° C., is subtracted from D, the count on the new agar at 32° C., the result is the effect on the count of changing the agar at 32° C. This may be be expressed as (D – B).
    c. If there are present any organisms requiring both that the

agar be enriched and the temperature of incubation be lowered before growth will occur, then (D − B) will be greater than (C − A), and the difference between these two values will give the number per milliliter of such organisms present.

d. Then the number of organisms of class 4 is given by (D − B) − (C − A), which is equivalent to (D + A − B − C). Thus, the number of organisms of this class was determined by adding D and A, and from that sum subtracting both B and C.

Table 2 shows the result of making the calculations described in the preceding paragraph. The four columns of figures have the same significance as in table 1.

TABLE 2

Numbers per milliliter of four classes of organisms in 100 lots of milk when raw and after pasteurization by three methods, when determined on two agars and at two temperatures of incubation

Treatment of milk	Class number	Description of class of organism	Number per milliliter	Per cent of number growing on old agar at 37° C.	Per cent of number growing on new agar at 32° C.	Per cent of number in raw milk (or per cent of thermodurics)
Raw	1	Grow on old agar at 37° C.	72,830	100.0	57.4	....
	2	Require 32° C. but not new agar	28,400	38.9	22.4	....
	3	Require new agar but not 32° C.	17,710	24.3	14.0	....
	4	Require both new agar and 32° C.	7,880	10.8	6.2	....
Pasteurized in the plant at 61.6° C. for 30 minutes	1	Grow on old agar at 37° C.	6,725	100.0	26.5	9.2
	2	Require 32° C. but not new agar	7,044	104.7	27.7	24.8
	3	Require new agar but not 32° C.	8,320	123.1	32.8	46.9
	4	Require both new agar and 32° C.	3,306	49.1	13.0	41.9
Pasteurized in the laboratory at 61.6° C. for 35 minutes	1	Grow on old agar at 37° C.	4,806	100.0	21.9	6.5
	2	Require 32° C. but not new agar	6,771	140.8	30.9	23.8
	3	Require new agar but not 32° C.	7,420	154.4	33.8	41.8
	4	Require both new agar and 32° C.	2,935	61.0	13.4	37.2
Pasteurized in the laboratory at 71.6° C. for 16 seconds	1	Grow on old agar at 37° C.	5,319	100.0	24.3	7.3
	2	Require 32° C. but not new agar	7,249	136.2	33.2	25.5
	3	Require new agar but not 32° C.	7,193	135.2	32.9	40.6
	4	Require both new agar and 32° C.	2,092	39.2	9.6	26.5

DISCUSSION

Examination of the data in tables 1 and 2 would seem to justify the following conclusions:

1. Either lowering of the temperature of incubation from 37° C. to 32° C., or enriching the agar, or making both changes simultaneously, results in a higher bacteria count with either raw or pasteurized milk (table 1).
2. With either raw or pasteurized milk, changing both temperature of incubation and composition of the agar simultaneously results in a greater increase in count than making either change alone (table 1).
3. The per cent increase in count due to any of these changes in methods of enumeration is from two and one-half to five times as great with pasteurized milk as with raw milk (table 1).
4. The most logical explanation of this difference between raw and pasteurized milk would seem to be as follows:
    a. Only a few of the raw-milk organisms that can grow on the old agar at 37° C. are capable of surviving pasteurization. The three methods of pasteurization used gave 6.5, 7.3 and 9.2 as the per cent of thermoduric bacteria among these raw-milk organisms of Class 1 (table 2).
    b. Of the bacteria in raw milk which require that the temperature of incubation be reduced, or that the medium be enriched, or that both changes be made before growth is possible on the plates, relatively large percentages are thermoduric. The fourth or right-hand column of table 2 shows that, in the milk examined, there were from 24 to 46 per cent of thermoduric bacteria among these raw-milk organisms of Classes 2, 3 and 4.
5. The discussion immediately above means that changing from the old standard method of making plate counts in any one of the three ways studied results not only in higher total counts on both raw and pasteurized milk, but also results in a greater percentage of the organisms counted in the raw milk being classified as thermoduric. Thus, with all three methods of pasteurization used, the per cent of thermoduric bacteria (table 1) was more than twice as great when using the new agar at 32° C. as when using the old agar at 37° C.
6. The organisms requiring for their growth both that the old agar be enriched and that the temperature be reduced from 37° C. to 32° C. (Class 4 in table 2) are obviously capable of growing only under rather restricted environmental conditions. That being the case, it might be expected that they would not be as numerous as other organisms capable of growing under a wider range of conditions. It is

interesting to note (table 2) that organisms of Class 4 actually do form the smallest of the four classes in both the raw milk and the milk pasteurized by each of the three methods.

7. Laboratory high-temperature, short-hold pasteurization tends to give higher counts than does laboratory low-temperature, long-hold pasteurization (table 1).
8. As the method of enumeration is changed so as to give higher and higher counts, the difference in count between milk pasteurized by the two laboratory methods decreases.

There is in the literature evidence tending to support conclusions one to five above (9, 10, 11, 12), and also conclusion seven (13, 14, 15, 16, 17, 18, 19, 20, 21, 22). It seems probable, therefore, that these conclusions have a fairly broad applicability to many milk supplies, although an effective campaign to reduce to a very low point the number of thermoduric bacteria in a given milk supply might alter the picture somewhat. Any idea as to how broadly conclusions six and eight could be applied to other milk supplies must await further investigation by other workers and with other milk supplies.

It should be pointed out that the calculations on which table 2 is based imply the assumption that there were in the samples no organisms growing at 37° C. but not at 32° C., or growing on the old but not on the new agar. That assumption, of course, is not entirely justified, but how many such organisms occurred in the samples examined could not be determined by the methods used.

The control and elimination from milk supplies of thermoduric bacteria is costing the dairy industry and the milk producer large sums of money. The problem assumes even greater magnitude when Departments of Health *lower* the number of bacteria they will allow in pasteurized products, as has been done recently in several localities. To illustrate this, a reduction during 1940 in allowable bacteria count in pasteurized milk from 50,000 to 30,000, or a decrease of 20,000, appears to be a reduction of 40.0 per cent. However, if this is compared with June, 1939, before the new agar became official, the picture is considerably changed. Table 1 shows, for the average of 100 comparisons on commercially pasteurized milk, an increase of from 6,725 to 15,045 bacteria per milliliter, or 123.7 per cent in changing from the old to the new agar at 37° C. At this rate, a sample that would have shown a count of 50,000 bacteria per milliliter in June of 1939 would have a count of 111,850 at present. Therefore, enforcement of a standard calling for a maximum of 30,000 per milliliter at the present time actually means a reduction of 73.1 per cent as compared with two years ago. If the temperature of incubation is also changed, another increase in count, of even greater magnitude, will occur, which will increase still more the difficulty and expense of keeping the bacteria count of pasteurized milk at very low levels.

SUMMARY

Data on 100 lots of milk examined both before and after pasteurization shows that lowering the temperature of incubation for plate counts from 37° C. to 32° C. or changing from the old standard agar to tryptone glucose extract milk agar, or making both changes simultaneously, not only results in higher counts on both raw and pasteurized milk, but also results in a higher percentage of the organisms counted in the raw milk being classified as thermoduric. Three different methods of pasteurization all gave this same result.

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THE DETERMINATION OF FAT IN THE PRESENCE OF FREE FATTY ACIDS. II. DIFFERENCES IN THE BEHAVIOR OF INDIVIDUAL ACIDS IN THE MOJONNIER TEST

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In a previous report (8) we demonstrated that approximately 24 per cent of a mixture of fatty acids which resembled completely hydrolyzed butterfat was extracted in the Mojonnier test and determined as butterfat. This substantiated earlier reports (1, 4) of low Mojonnier fat tests in rancid dairy products, and indicated, also, that not all of the free fatty acid was retained in the ammoniacal layer. In those experiments, we used a mixture of free fatty acids which resembled that which would have resulted from the hydrolysis of butterfat by a *non-specific* lipase. Davies (2) states that ". . . the lipases are . . . specific in their action . . . thus lipases from various sources will show different rates of liberation of free fatty acids and a different distribution of such acids, although it is recognized that it is the unsaturated acids, *e.g.*, oleic acid, which are liberated in greatest amount. . . ." For this reason, data on the recovery of individual fatty acids would be of some value in interpreting the decrease in fat test when examining rancid samples by the Mojonnier method.

EXPERIMENTAL

Quantities of a butter oil, which had been prepared from a fresh, high quality, unsalted, sweet-cream butter, and which contained 99.1 per cent of Mojonnier-extractable fat, were weighed into Mojonnier flasks. To each flask was added a weighed amount of *one* of the following fatty acids: butyric, lauric, myristic, palmitic, stearic, oleic. The stoppered flasks were then warmed in a water bath to melt the contents, shaken to mix the fat and acid, and then examined for "fat content" by means of the conventional Mojonnier test for butter (5). The data obtained in this manner are shown in table 1.

These data confirm our previous report that a certain fraction of free fatty acid is extracted and determined as butterfat when rancid butterfat is examined by the Mojonnier method for fat.

The quantity of free fatty acid which is extracted depends upon the nature of the fatty acid. Undoubtedly a number of more-or-less obvious

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TABLE 1

Mojonnier analyses of samples consisting of butter oil plus a free fatty acid

No.	A Fat. Corrected weight of Mojonnier extractable fat in butter-oil	B Fatty acid	C Total sample (A + B)	D Recovered by extraction	E Fatty acid not recovered (C − D)	F Fatty acid not recovered $\frac{E \times 100}{B}$	G Average
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	%	%
				Oleic			
1	472.9	0.0	472.9	472.9	.	..	
2	379.0	107.6	486.6	408.8	77.8	72.3	
3	339.9	160.0	499.9	384.0	115.9	72.4	
4	195.5	271.6	467.1	269.3	197.8	72.8	
5	0.0	909.0	909.0	128.0	781.0	(86.0)*	72.5
				Stearic			
6	349.4	0.0	349.4	349.4	.	. ...	
7	279.5	29.7	309.2	285.7	23.5	79.1	
8	387.4	60.8	448.2	400.9	47.3	77.8	
9	190.6	220.5	411.1	239.7	171.4	77.7	
10	0.0	177.1	177.1	38.6	138.5	78.2	78.2
				Palmitic			
11	195.6	0.0	195.6	197.5	....	....	
12	469.4	0.0	469.4	470.0	.. ..	....	
13	375.8	67.1	442.9	390.4	52.5	78.2	
14	200.0	66.5	266.5	215.1	51.4	77.3	
15	275.0	114.3	389.3	302.4	86.9	76.0	
16	363.3	157.5	520.8	403.2	117.6	75.3	
17	256.2	177.3	433.5	297.8	135.7	76.5	
18	0.0	121.9	121.9	28.2	93.7	76.9	76.7
				Myristic			
19	352.0	0.0	352.0	352.0	....	....	
20	286.8	57.7	344.5	291.4	53.1	92.0	
21	182.8	311.7	494.5	208.0	286.5	91.9	
22	0.0	223.4	223.4	1.0	222.4	(99.6)*	92.0
				Lauric			
23	278.2	0.0	278.2	278.2	.	....	
24	345.1	25.5	370.6	346.7	23.9	93.7	
25	316.7	119.6	436.3	325.3	111.0	92.8	
26	358.9	152.8	511.7	368.6	143.1	93.7	
27	0.0	169.6	169.6	1.2	168.4	(99.3)*	93.4
				Butyric			
28	369.5	67.6	437.1	369.3	67.8	100.3	
29	0.0	182.3	182.3	0.6	181.7	99.7	100.0

* Values in parentheses are excluded from the averages for reasons given in text.

factors are instrumental in causing these differences. Certain of these factors have been studied and will be discussed in turn; *viz.*, the hydrolysis of easily-dissociated ammonium soaps, the extraction of fatty acids thus liberated, and the loss of these extracted acids by volatilization.

The presence of ammonia in the ether extracts *before* evaporation may be demonstrated by means of Nessler's reagent. However, since ammonia is found, also, in the ether extract of a lipid-free control, the ammonia is not necessarily extracted in the form of an ammonium soap, and it is probable that the positive Nessler test may be traced to the extraction of some ammonia by the ether. Since no ammonia can be found in the lipid residue *after* evaporation of the ether, the volatile ammonia is probably driven off when the ether is evaporated. In this connection, one should not ignore the possibility that the ammonium soap was extracted but was decomposed by the heating afterward. Even $(NH_4)_2SO_4$ is reported to lose ammonia at 120° C. and, in some cases, even as low as 80° C. (7).

Since the residues in the fat dishes do not contain ammonia, it is evident that the fatty acid increment is free fatty acid and not the ammonium soap. This would be expected since these soaps are salts of the weak base, ammonium hydroxide, and the weak fatty acids. Extensive hydrolysis probably occurs in aqueous solution with the liberation of free fatty acids.

Indirect evidence for such an hydrolysis might be obtained by comparing the behavior of ammonium hydroxide with the behavior of a much stronger base. Lithium hydroxide seemed suitable for this comparison. It is a much stronger base than ammonia (the normal solutions are dissociated to the extent of 63 per cent and 0.4 per cent respectively (3)); and it yields soaps which are insoluble in ether but quite soluble in methyl alcohol (6).

These two bases were compared by running a series of analyses of cottonseed oil-oleic acid mixtures. The ammonia method yielded high results (approximately 27 per cent of the oleic acid was recovered), the lithium method gave the theoretical value for neutral fat alone. This difference is attributed to differences in the degree of hydrolysis of the respective soap solutions rather than to differences in the solubility of the soaps because we were unable to detect the presence of ammonia in the extracted lipid residues by means of Nessler's reagent.

We do not, however, advocate the substitution of lithium hydroxide for ammonium hydroxide in the Mojonnier test because troublesome emulsions may be formed and because the strongly alkaline lithium hydroxide may cause the saponification of some kinds of fat.

A further study of the data of table 1 shows that the recovery of fatty acid in the Mojonnier test varies with the molecular weight of the acid. This may be explained, in part, on the basis of differing distributions between the two solvents, and to differing volatilities of the extracted fatty acids. The solubility of fatty acids in water decreases with increasing

molecular weight. It should be expected, then, as is shown in table 1, that in the distribution of these acids between water and ether, a greater percentage of the acids of high molecular weight will be extracted by the ether than of acids of low molecular weight.

The acids of low molecular weight are more volatile than those of high molecular weight. The influence of volatility in reducing the recovery of certain free acids was shown by the following experiments:

Quantities of fatty acids and of butter oil were weighed directly into tared fat dishes; 50 ml. of ethyl ether and 50 ml. of petroleum ether were added and mixed with the lipids. The mixture was then heated on the Mojonnier fat plate to drive off the ethers, dried in the fat oven, cooled and weighed as in the conventional Mojonnier procedure. Some data obtained in this manner are recorded in table 2.

TABLE 2

The loss of fatty acid by volatilization during evaporation of the ethers in the Mojonnier fat test

No.	A	B		C	D	E	F
	Fat. Dry weight of butter oil	Fatty acid		Total sample (A + B)	Recovered after evaporation of ether	Fatty acid not recovered	
		Name	Weight			(C − D)	$\frac{E \times 100}{B}$
	mg.		*mg.*	*mg.*	*mg.*	*mg.*	%
101	555.0	....	0.0	555.0	555.1	....	....
102	419.7	Oleic	43.8	463.5	458.4	5.1	12.0
103	419.7	Oleic	143.0	562.7	560.1	2.6	2.0
104	0.0	Oleic	194.1	194.1	168.2	25.9	13.0
105	443.3	Stearic	150.1	593.4	593.1	0.3	0.2
106	0.0	Stearic	145.1	145.1	143.8	1.3	1.0
107	421.0	Palmitic	41.1	462.1	459.0	3.1	8.0
108	0.0	Palmitic	52.3	52.3	30.7	21.6	42.0
109	494.7	Myristic	225.8	720.5	716.1	4.4	2.0
110	0.0	Myristic	217.8	217.8	193.3	24.5	11.0
111	486.5	Lauric	76.7	563.2	542.7	21.5	28.0
112	0.0	Lauric	51.1	51.1	0.0	51.1	100.0
113	0.0	Butyric	182.3	182.3	0.6	181.7	100.0
114	0.0	....	0.0	0.0	0.1	....	....

These data show that the recovery of fatty acids in the Mojonnier test is influenced by the volatility of the acid. They also show that the volatility of the acids is reduced considerably by the presence of neutral fat. This probably accounts for the apparent discrepancies recorded in table 1; *i.e.*, the high results in "amount of fatty acid not recovered" from samples 5, 22 and 27. In each of these cases, no neutral butterfat was present in the original sample. In those samples, a proportionately greater quantity of the extracted free acid was volatilized during the ether evaporation and, in this manner, a greater quantity of the fatty acid was "not recovered."

SUMMARY

The Mojonnier test does not recover equal percentages of the various free acids which may be present in the butterfat. This is due, in part at least, to variations in the amount of different acids volatilized when the sample is heated to remove the ether.

It seems probable that the recovery of free acids by the Mojonnier method can be traced to hydrolysis of the ammonium soaps with the subsequent extraction of the liberated acid. The degree of hydrolysis would depend upon the nature of the acid, but it might be reduced in all cases by the substitution of a stronger base for the ammonia. Lithium hydroxide offers some promise, but its saponifying action must be studied more carefully before it can be recommended.

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BUTTERFAT AND SILAGE CAROTENOIDS*

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Virtanen (12), Uuranen (11) and Peterson *et al.* (7, 8) observed that the apparent carotene content of silage prepared with acids was in many cases greater than that of the fresh material from which it had been made. The reason for these high values was found by Quackenbush, Steenbock and Peterson (9) to be the presence of pigments produced from xanthophyll by the action of silage acids. These pigments were carried along with carotene in the petroleum ether-ethanol procedure but could be separated from the carotene and from each other by chromatographic means. In some silages they made up 40 per cent of the so-called carotene, but unlike carotene they had no vitamin A potency.

It was the object of the present experiments to determine the extent to which the non-carotene pigments in "acid silages" would appear in butter produced from the milk of cows fed these silages. If these pigments were secreted into milk, they would be calculated as carotene and would give a false value to the vitamin A potency of the milk.

It was observed by Palmer and Eckles (6) that both carotene and xanthophyll occurred among the pigments of butter, but by far the greatest portion of the total pigment was carotene. Karrer and Schöpf (5) identified chromatographically lutein and zeazanthin as well as carotene in butter. Gillam and Heilbron (3) found β-carotene and small amounts of α-carotene, kryptoxanthin and lycopene in the butter fat from cows which had received rations containing these pigments.

It is evident as stated by Strain (10) that the carotenoids of butter are dependent upon those in the ration of the cow, but they are present in different proportions in butter probably because carotene is much more readily absorbed than the other pigments.

EXPERIMENTAL

Butter was made in the winter of 1939 and again in 1940 from the milk of two groups of cows which had received respectively phosphoric acid alfalfa silage and molasses alfalfa silage for four months. Butter produced on summer pasture was used each year as a control. The butter samples were stored at a temperature below 0° C. and when required for analysis were melted and freed from water and solid matter by filtering through absorbent cotton. They were saponified under nitrogen, and the non-

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saponifiable matter was extracted with peroxide-free ether. The ether solution was washed several times with ice water, dried by freezing out the water with dry ice, evaporated to dryness in vacuum, and the residue taken up in 2 to 3 cc. of purified petroleum ether (Skelly-solve B). The solution was kept cool at all times and evaporated under vacuum in order to minimize isomerization as reported by Carter and Gillam (1) and by Zechmeister and Tuzson (13). The resultant solution was chromatographed through $CaCO_3$ or MgO columns. In most of the work $CaCO_3$ proved to be superior.

The pigments were fractionated as follows: The petroleum ether solution was forced into a uniformly packed column of adsorbent by means of air under a pressure of 15–25 pounds. The column was then washed with petroleum ether under continued pressure until the bands of carotene, acid-formed pigments and xanthophylls had separated. The bands of carotene and acid-formed pigments were washed through and collected separately. Then the xanthophyll bands were washed through with a 10 per cent solution of absolute alcohol in petroleum ether and collected together. The pigments were determined quantitatively in three groups: 1) carotene, 2)

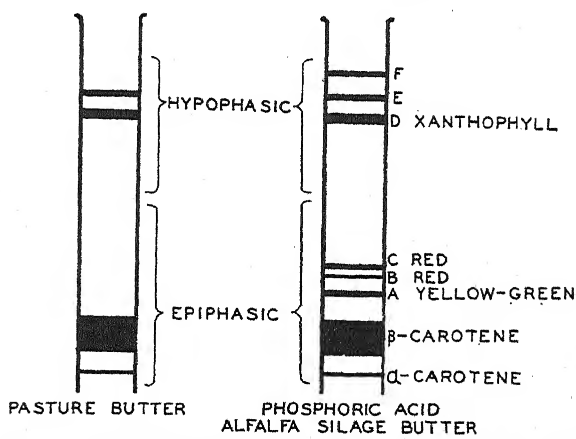

FIG. 1. Chromatograms of carotenoid pigments in butter fat.

pigments A, B, C (the so-called acid-formed pigments, Fig. 1), and 3) pigments D, E, F (the hypophasic xanthophyll pigments). The total pigment was first calculated as carotene by determining the intensity of spectral absorption in petroleum ether solution with a 440 mμ filter in the Evelyn photelometer. After chromatographing, each fraction was read at 440 mμ in petroleum ether and expressed as carotene. The sum of these values was found to account for 95 to 97 per cent of the original values. Only the pigments which it seemed most important to identify were separated from the mixture.

To reveal the phasic distribution of the pigments a petroleum ether solu-

tion of the non-saponifiable fraction was extracted with 90 per cent methanol and the resulting two fractions were chromatographed from petroleum ether solution as before. In the case of the silage butters the carotene and the acid-formed pigments were found to be epiphasic while the xanthophylls were hypophasic.

RESULTS

The pigments which had been isolated chromatographically were identified so far as possible by determining their absorption maxima. The spectra were photographed in either petroleum ether or carbon bisulfide, with the use of a Hilger spectrograph. The wave lengths of the band maxima are given in table 1. The red acid-formed pigments (pigments B and C, Fig. 1) did not give distinct bands even in concentrated solution. In both petroleum ether and in carbon bisulfide, absorption was general over a wide range and the values given are, therefore, only approximate. It is possible that these pigments are still mixtures of degradation products.

TABLE 1

Absorption maxima of pigments isolated from butter

Pigment	In petroleum ether (mμ)	In carbon bisulfide (mμ)
α-Carotene { isolated	477, 444, 416	
α-Carotene { accepted value	478, 447.5	
β-Carotene { isolated	483, 452, 425	519, 482, 450
β-Carotene { accepted value	483.5, 452, 426	521, 485.5, 451
Yellow-green, acid-formed, pigment A	454, 428, 403	489, 454, 428
Red, acid-formed, pigment B	(495, 459, 425)?	....
Red, acid-formed, pigment C	(453, 433)?	(480)?
Xanthophyll { isolated		508, 473, 445
Xanthophyll { accepted value		508, 475, 445

Traces of α-carotene were found in the butters only when relatively large amounts of butter (100 gms.) were used in one analysis. This finding agrees with that of Gillam and El Ridi (2) who reported the α-carotene content to be less than 0.3 per cent of the β-carotene content in the butters which they examined.

The amounts of the pigment in each of the three groups for the phosphoric acid and the molasses alfalfa silage butters and for pasture butter are given in table 2. These figures are averages of the values obtained in 1939 and in 1940. From this table it appears that: 1) There is more carotene in pasture butter than in silage butter because of the higher carotene content of the pasture. However the ratio of carotene in butterfat to that in forage shows that carotene is absorbed just as well from silage as from pasture. 2) There are no acid-formed pigments in pasture and thus none

in pasture-butter. There are, as shown by Quackenbush *et al.* (9), more acid-formed pigments in acid silage than in molasses silage and, as would be expected, there are more acid-formed pigments in butter produced on phosphoric acid silage than on molasses silage. 3) While the acid-formed pigments seem to be rather readily absorbed and transferred into the butter-fat, the xanthophyll pigments appear to be so poorly absorbed that there is about the same amount of xanthophyll pigments in butters irrespective of the amount in the ration.

TABLE 2

Amounts of pigments found in butter (2 year averages)

Type of butter	Pigments in butter fat				(b) Carotene in forage	Ratio a/b
	Total	Xanthophylls	Acid-formed	(a) Carotene		
	γ/gm.	γ/gm.	γ/gm.	γ/gm.	γ/gm.	
Pasture	11.0	2.2	0.0	8.8	250	0.035
Molasses alfalfa silage	9.0	2.2	0.7	6.1	140	0.043
Phosphoric acid alfalfa silage	8.5	2.0	1.0	5.5	120	0.046

Table 3 gives the distribution of the three groups of pigments expressed as per cent of the total pigments. In an analysis of the phosphoric acid alfalfa silage butter the 12 per cent of the acid-formed group was found to be made up of 3.4 per cent of the greenish-yellow pigment A and 8.6 per cent of the red pigments B and C.

TABLE 3

Distribution of pigments in butter (2 year averages)

Type of butter	Percentage of total pigments		
	Carotene	Acid-formed pigments	Xanthophylls
	%	%	%
Pasture	80	0	20
Molasses alfalfa silage	68	8	24
Phosphoric acid alfalfa silage	65	12	23

In order to obtain true values for the carotene content of milk the non-carotene pigments should be removed before the reading is taken. It would appear that the diacetone alcohol method of Hegsted *et al.* (4) might be applicable to milk and butter as well as to silage for the separation of the carotene and acid-formed pigments.

The above data should not be interpreted as denying the value of these forages for increasing the carotene content of winter milk, since only a part of the increased color is due to non-carotene pigments. Although no data

were obtained for butters produced by cows on hay-corn silage rations, it is probable that the percentage of non-carotene pigments in such butters would be as high as when the cows are fed molasses silage. Corn silage usually has a lower pH than molasses silage and hence a larger proportion of acid formed pigments would probably be present and presumably transmitted to the milk.

SUMMARY

Besides carotene and xanthophyll, pigments formed by the action of acids in silage are carried over into the butter fat of the milk. The usual methods of carotene analysis on butter fat do not distinguish between carotene and non-carotene pigments. By chromatographic methods the distribution of pigments in butters from cows on different forages was found to be as follows: pasture, 80 per cent carotene, 20 per cent non-carotene; molasses alfalfa silage, 68 per cent carotene, 32 per cent non-carotene; phosphoric acid alfalfa silage, 65 per cent carotene, 35 per cent non-carotene.

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MENSTRUATION FREQUENCY AND ITS RELATION TO CONCEPTION IN DAIRY CATTLE[1]

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Among mammals, there is a marked variation in regard to menstruation and its relationship to conception. Examination of the literature reveals that the time in the sexual cycle at which menstruation takes place is well established in the different species but there is a marked individuality that is observed for each one and sometimes even within the species.

The human being (1, 7) is reported to have an exceptionally long menstruation period which lasts from two to eight days with an average of five days. If the occurrence is regular, the beginning of the menstrual period is half way between successive ovulations and is closely associated with estrus. Marshall (5), Howell (4), Schmaltz (7), and Reynolds (6), all agree that in the human, the mucous membrane thickens to several times normal as pre-menstrual congestion takes place. Although menstruation in the human being is a phenomenon of the uterus, and blood will escape only from the surface of that organ, the other reproductive organs share to some extent the vascular congestion exhibited by the uterus during this period. The congested capillaries of the uterus break down or rupture in the superficial regions of that organ. The blood discharged at menstruation may be due to these small capillary extravasations and also to a process of diapedesis or seepage made possible by the congestion. Sometimes, when menstrual flow is very profuse in the human, there may be a considerable loss of surface epithelium. The vessels in the deeper tissue remain intact and none of the fluid is found free in the deeper tissue of the stroma. Howell (4) states that menstruation is a sign that fertilization has not taken place from the previous ovulation but Schmaltz (7) gives evidence that it is possible to have a certain amount of menstrual flow following fertilization.

Usually the experienced dog breeder knows that a bitch will, in most cases, have a pronounced flow of blood during the period of proestrus which usually lasts about ten days. As a rule, the bitch will not take the dog until bleeding has ceased and the best time for successful coition is soon after the cessation of this flow. Marshall (5) and Schmaltz (7) agree with this common view.

According to Marshall (5) occasionally blood has been observed in the mare's proestrus discharge but is not generally present. He also states that the ewe menstruates very little and usually shows no external signs. Very

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rarely, a small amount of bloody mucous is observed during proestrus. The sow likewise usually does not show any external signs but occasionally a bloody mucous flow has been observed during proestrus.

The cow (2, 3, 9) is reported to menstruate about two days after heat. Hammond (3) presents evidence to show that in the cow the menstrual fluid comes from both the vagina and uterus. He expresses the opinion that the blood flow in the vagina comes from the region situated just above the urethra and that in the uterus there is special engorgement of the placental areas; but bleeding can come from the surface of any part of these two organs.

A few authors express the opinion that if a heifer or cow menstruates a few days after service it is an indication that conception did not take place, but in most cases they present no definite data to support their belief. W. W. Williams (9) states "The non-pregnant cow ordinarily menstruates about the 48th hour after heat subsides. A little blood-tinted mucus stringing from the vulva at this time, or upon the cow's tail, indicates that the service has been ineffective. The complete absence of any bloody discoloration of the vaginal mucus suggests that the cow has conceived especially if it has previously been noted that the cow menstruated normally at other periods."

Another author, W. L. Williams (8), writes, "Within 24 to 48 hours after a cow has been bred she may menstruate. The sanious discharge emanating from the vulva may adhere to that organ, the tail and adjacent parts. If the cow has been bred and conceived, it is doubtful if there will be menstruation following. If she fails to conceive, menstruation is quite certain to occur. In many cases of serious sterility, the volume of menstrual blood is very great. Fertilization appears to inhibit menstruation, but menstruation may occur in spite of conception. The absence or presence of menstruation must not be accepted as final proof of conception or non-conception. It is, however, a valuable sign, and should always place the breeder and veterinarian on guard, with a rather definite expectation that the animal which has menstruated after breeding will again be in estrum in due course of time."

Hammond (3) carefully observed four heifers after service to a fertile bull and states that all four menstruated at the normal time a few days later. Three of these had become pregnant and subsequent bleeding did not occur in these three. He refers to the fact that it is a common belief among herdsmen that bleeding does not occur two days after heat if fertilization takes place.

EXPERIMENTAL PROCEDURE

Because of the widespread belief among livestock men that menstruation in cattle occurring after service is an indication that conception did not take place, and because of the conflicting opinions expressed in the litera-

ture, it seemed that the problem was worthy of consideration. As a result, data have been collected in the University of Nebraska dairy herd as to the occurrence of menstruation following estrus in heifers and cows. The study covered the years 1937 to 1940 and included representatives of the Jersey, Guernsey, Holstein, and Ayrshire breeds. As used in this study, menstruation refers to the external discharge of a bloody fluid, usually mucus mixed with blood, from the genital tract following estrus.

A group of 100 heifers and a group of 100 cows were studied to determine the frequency of the occurrence of menstruation following estrus when they were not served. For comparison, two similar groups consisting of 100 cows and 100 heifers were inseminated during estrus and the frequency of subsequent menstruation and conception recorded. The amount of menstrual fluid discharged was also noted and given a rating of slight, moderate, and pronounced. Observations were made at six regular intervals each day for five days following estrus and any external signs of a blood-tinted discharge were noted.

PRESENTATION OF RESULTS

Table 1 presents data on the occurrence of menstruation following estrus in open and bred heifers and cows and its relation to conception.

TABLE 1

Occurrence of menstruation following estrus in open and bred heifers and cows and its relation to conception

Group	Females	After estrus: Females menstruating within 5 days	Females conceiving	Females conceiving and menstruating		Females not conceiving	Females not conceiving but menstruating	
	no.	*no.*	*no.*	*no.*	%	*no.*	*no.*	%
Heifers (open)	100	100	......	......	....	......	......	....
Cows (open)......	100	61	......	......	....	......	......	....
Heifers (bred)	100	81	61	52	85.25	39	29	74.36
Cows (bred)......	100	61	72	50	69.44	28	11	39.29

Menstruation was observed in everyone of the 100 heifers not bred during estrus. In the group of 100 cows not bred during estrus, a total of 61 had a menstrual discharge. Of the lot of 100 heifers bred at estrus, a total of 81 menstruated. Sixty-one heifers conceived and of these, 52 or 85.25 per cent menstruated within five days while 9 or 14.75 per cent showed no evidence of menstruation. From the 39 heifers which did not conceive after breeding, 29 or 74.36 per cent menstruated while 10 or 25.64 per cent showed no signs of menstruation. In the group of 100 cows bred at estrus, 61 menstruated within five days. Seventy-two cows conceived and of these 50 or 69.44 per cent menstruated while 22 or 30.56 per cent did not men-

struate. Of the 28 cows which did not conceive after breeding, 11 or 39.29 per cent menstruated and 17 or 60.71 per cent did not menstruate.

The estimated amount of menstrual fluid and the time after estrus when this was discharged was recorded and these data are presented in table 2.

TABLE 2

Time of menstruation and amount of menstrual fluid

Group	Females grouped according to estimated quantity of menstrual fluid			Females grouped according to time of menstruation			
				Days after estrum			
	Slight	Moderate	Profuse	1st	2nd	3rd	4th
	no.	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*
Heifers (open)	32	59	9	4	82	12	2
Heifers (bred—no conception)	11	15	3	1	16	8	4
Heifers (bred—conceived)	17	29	6	6	39	7	...
Cows (open)	22	31	8	8	49	4	...
Cows (bred—no conception)	4	7	...	...	5	6	...
Cows (bred—conceived)	22	28	...	8	34	8	...
Total	108	169	26	27	225	45	6

In the above table, the data indicate that for the 303 individuals that menstruated, 108 or 35.64 per cent had a slight amount, 169 or 55.78 per cent a moderate, and 26 or 8.58 per cent a profuse amount of menstrual fluid. The second part of table 2 in which the females were grouped according to time of menstruation in days after estrus shows that 27 or 8.91 per cent menstruated on the first day, 225 or 74.26 per cent on the second day, 45 or 14.85 per cent on the third, and 6 or 1.98 per cent on the fourth day following estrus.

DISCUSSION

This study offers evidence that the common belief among livestock men and veterinarians that a heifer or cow which menstruates a few days after service did not conceive is erroneous. These data show to what a surprising degree this supposition is in error. The data presented reveal that of the 100 heifers which were bred, 61 conceived and that 85.25 per cent of those conceiving also menstruated. A group of 100 cows bred resulted in 72 conceptions and 69.44 per cent of those conceiving also menstruated. It was found that the frequency of menstruation in heifers is greater than in older cows. This may be due to the fact that heifers as a rule are more excited at heat than are older cows which may result in the genital organs becoming more engorged with blood. The genital organs of the cow also are longer and extend further forward and occupy more of an abdominal position as compared to the pelvic position in heifers and this may have a tendency to prevent the flow of the menstrual fluid from the vulva of some

of the older animals. Another point that might be mentioned is that the amount of menstrual fluid is possibly dependent on the thickness and toughness of the endometrium. This will vary with age and number of calvings and there will be some variation between individuals. The data presented indicate that of the 303 females that menstruated, 225 or 74.26 per cent passed off the menstrual fluid on the second day following estrus.

SUMMARY

Observations as to the external evidence of the occurrence of menstruation were made at six different times daily on each of five days following estrus, for four lots of dairy females each consisting of 100 animals. One hundred heifers and an equal number of cows were observed after estrus, when breeding did not occur, and 100 heifers and 61 cows showed evidence of menstruation. Two similar groups of 100 heifers and 100 cows were bred during estrus. Of the 100 heifers bred at estrus, 81 menstruated. Sixty-one of the heifers conceived and 52 or 85.25 per cent of those conceiving also menstruated while of the 39 that did not conceive, 29 or 74.36 per cent menstruated. Of the 100 cows bred at estrus, 61 menstruated. Seventy-two of these cows conceived, and 50 or 69.44 per cent of those conceiving also menstruated, while of the 28 that did not conceive, 11 or 39.29 per cent menstruated. The data do not indicate any definite relationship between breeding and conception as affecting menstruation. When external evidences of menstruation were observed a total of 303 of the 400 individuals menstruated and 225 or 74.26 per cent showed this menstrual discharge on the second day following estrus.

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FINAL REPORT OF COMMITTEE ON METHODS OF DETERMINING THE CURD TENSION OF MILK

The curd tension of milk, while probably not wholly satisfactory as a measure of digestibility and suitability of milk for infant use, nevertheless has been tacitly accepted as an indication of these characteristics. The recent action of the Council on Foods of the A.M.A. in "accepting" homogenized milks with soft curd claims and establishing a limit of 20 grams tension for such milks has further added to the repute of the curd tension value. Some municipalities have set, or are contemplating establishing, curd tension standards for homogenized milk, the production of which is increasing rapidly. It seems particularly desirable, therefore, to publish the procedure for making curd tension determinations which was approved at the Thirty-sixth Annual Meeting of the American Dairy Science Association, June 1941.

DETERMINATION OF THE CURD TENSION OF MILK

Reagent

The coagulant consists of .08 N hydrochloric acid to which U.S.P. (1 : 3000) dry pepsin has been added at the rate of 450 mgms. per 100 ml. This solution may be kept under refrigeration and away from light for a period of not more than 10 days without losing its effectiveness but, in case of doubt, results obtained with it must be checked against results obtained using a freshly prepared coagulant.

Apparatus

The instrument used in measuring the curd tension shall indicate the grams of force* necessary to cut the coagulum, obtained as described below, using the standard type of knife. The motion of the knife or the vessel containing the coagulum shall be automatic and at the rate of one inch in 7 to 8 seconds, obtained by lowering the knife or by raising the vessel containing the coagulum. The sensitivity of the instrument shall be such that readings can be made to an accuracy of $\pm$ 1.0 gram.

Standard Knife

The knife used as a part of the instrument for measuring the curd tension shall consist of eight radial blades each having $\frac{9}{16}$ inches of lineal cutting edge and being .020 inches thick, spaced equally and enclosed by a circular or ring blade, $1\frac{3}{4}$ inches outside diameter, $\frac{3}{16}$ inches high and .031 inches thick. The radial blades shall be attached to the inside of the circular or ring blade and extend upward from it a distance of $2\frac{1}{16}$ inches,

* Submarine Signal Company Curd Tension Meters made prior to July 1941 do not read in grams. Readings may be converted to grams, however, by adding 10 per cent (multiplying by 1.10).

being reduced to a width of 5/32 inches above the circular or ring blade. Their upper ends shall be curved inward and attached to a central spindle $\frac{5}{16}$ inches in diameter. The lower or cutting edge of the circular or ring blade shall be tapered from the outside at an angle of 30 degrees to the knife axis, to a dull knife edge. The lower or cutting edges of the radial blades shall be tapered, on each side, at an angle of 15 degrees to the knife axis, to a dull knife edge. The cutting edges of the ring blade and the radial blades shall lie approximately in the same plane, deviating not more than 1/32 inches. The total linear cutting edge of the knife shall be 9.8 inches $\pm$ 0.10 inches. All joints shall be mortised and soldered smoothly and the knife constructed of a non-corrosive metal.

Coagulation Vessel

The receptacle used for coagulating the milk shall be a heavy walled jar 3 to 4 inches high (inside) and having an inside diameter of $2\frac{3}{8}$ inches $\pm \frac{1}{8}$ inch. The jars listed below have been found to meet these specifications satisfactorily and undoubtedly others will also be found which conform.

W. M. Welch Scientific Company, Chicago—Museum jar No. 4612A (8 oz.)
Central Scientific Co., Chicago—Museum jar No. 10396 ($7\frac{1}{2}$ oz.)
Owens Illinois Glass Co., Toledo—Mayonnaise jar (8 oz.)

Sample Pipette

The pipette used for measuring and adding the milk sample to the jar containing the coagulant shall be one made to deliver 100 ml. which has had the tip removed and which empties water by gravity in approximately 4.5 seconds.

Water Bath

A suitable water bath shall be used to temper the jars containing the coagulant before the sample of milk is added and to hold the milk and coagulant at the proper temperature during the coagulating period. The water level shall come up on the outside of the jar to a point no lower than the milk and coagulant level on the inside of the jar. The volume of water shall be such that the temperature does not change more than 1.0° F. during the 10-minute period of coagulation and should preferably be agitated.

Procedure

Introduce 10 ml. of the coagulant into the coagulating vessel and set the vessel in the water bath, having previously adjusted the temperature of the water to 95° F. The vessel containing the coagulant should be tempered for no less than 3 minutes before introducing the milk sample.

The undiluted milk to be tested is tempered to 95° F. or 96° F. as experience dictates being careful not to exceed 100° F. in the process. The tempering should be accomplished in a five-minute period immediately preced-

ing the pipetting of the sample. Introduce 100 ml. of the sample into the coagulating vessel using the tipless 100 ml. pipette. This is to be accomplished by holding the pipette vertically over the center of the vessel and blowing the sample from the pipette as rapidly as possible. No further mixing of the vessel contents is to be employed and the vessel should not be disturbed in the water bath. Place a watch glass or other suitable covering over the vessel immediately.

The contents of the coagulating vessel are held at 95° F. $\pm$ 1.0° for 10 minutes, the time in the water bath being adjusted so that the cutting of the curd by the curd tension instrument occurs at the expiration of 10 minutes $\pm$ 30 seconds from the time the sample was placed in the coagulation vessel.

The curd tension reading is the maximum reading which is obtained at the moment the knife penetrates the surface of the coagulum. The test shall be made in duplicate or replicate and results to be acceptable must not deviate more than 5 per cent from the average. The average of the results so checking constitutes the curd tension of the sample.

Remarks

The curd tension value of milk should not be considered an absolute index of its digestibility nor of its suitability for infant feeding purposes. The values do, however, correlate in a general way with these properties and the determination appears to be the best available, simple method for the purpose.

The method is conventional and the procedure must be followed closely otherwise considerable variation in readings may result. One very important factor is that of securing *uniform* mixing of milk and coagulant with *promptness* so that the mixture will be quiescent before coagulation commences.

Another factor which has been shown to somewhat influence results is the speed of warming the milk samples to the coagulating temperature. Where the milk is warmed very rapidly the results are slightly lower than where slow warming is practiced.

Adjusting the pH of milk for the purpose of making curd tension determinations is not a part of this method. Results obtained after such adjustments cannot be considered normal.

The determination of the curd tension of milk as here described is recommended for use with undiluted milk only. Some types of soft curd milk on dilution give results which cannot be considered meaningful.

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THE PRODUCTION AND USE OF CONCENTRATED SKIM MILK FOAM

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The foaming of skim milk during various manufacturing operations has long been a source of annoyance to dairy products manufacturers. Many reports deal with prevention and destruction of foam but apparently there have been few attempts to utilize the foaming property of skim milk. While fresh skim milk forms a relatively unstable surface foam, the results presented here will show that reconstituted dry skim milk or concentrated skim milk can often be whipped to a stiff white foam of high stability. Some effects of temperature and time of whipping, concentration of solids, and of some variations in manufacturing procedure upon skim milk foam production and stability are reported. A new food use for dry or concentrated skim milks which whip readily is indicated.

The foaming of milk has been studied by several investigators, a concise discussion being available in Fundamentals of Dairy Science (2). A recent study of Ansbacher, Flanigan, and Supplee (1) is concerned with the substances in milk which cause foam production. Sharp, Myers, and Guthrie (6) were unable to decrease the foaming capacity of skim milk by repeatedly foaming and removing the foam thus formed, nor did they find an accumulation of any major protein fraction in the foam. Studies on the effect of temperature upon the foaming of skim milk (3, 5) indicate that between the temperatures of 20° C. and 30° C. the foaming tendency is at a minimum. Foaming increases above and below this temperature range.

Evaporated milk can be whipped when it is maintained at low temperatures but since this product is manufactured from whole milk, its relatively high fat content inhibits the incorporation of a large quantity of air. Recently Leviton and Leighton (4) have discussed the depressing effect of fat upon skim milk foam.

EXPERIMENTAL

Foam was produced by whipping concentrated skim milk or reconstituted dry skim milk in a small electrically-operated mixer that maintained at high

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speed, 1,000 r.p.m. without a load. When skim milk powder was used, it was added to water in the mixer bowl and beaten at low speed for one minute before whipping at high speed. The time figures given with the data in this paper refer to the number of minutes the sample was whipped at high speed and do not include the one-minute mixing period. Most of the data were obtained by using reconstituted dry skim milks of 30 per cent solids. To prepare these milks 49-½ grams of dry skim milk were added to 110-½ cc. of water. An allowance of 3 per cent moisture was made for all dry milk samples. Different samples were whipped to secure the data for each time period. Percentage overrun was calculated as 100 times the difference between the weight of 175 cc. of milk measured before whipping and 175 cc. measured after whipping, divided by the weight of 175 cc. measured after whipping.

The stability of the foam produced by whipping was determined by observing the time required for the first drainage to appear in the bottom of a 160 cc. glass tumbler filled with the whipped milk. The limit of error in the whipping and stability tests was ± 5 per cent but the actual error in most cases was probably less than half of this figure.

Viscosity measurements were made on the foam drainage obtained from 3 to 6 hours after whipping. A McMichael viscosimeter was used and the wires were standardized with sugar solutions.

The temperature at which all whipping, drainage, and viscosity tests were conducted was 20° C. unless otherwise stated.

The dry skim milks used for the experiments reported in tables 1 and 2 were commercial products prepared for use in bread making. Fresh skim milk from the Beltsville Research Center was used to obtain the data of table 3. The eight samples of skim milk required for the experiments of table 4 and figure 1 were derived from various sources as indicated. On a basis of 9 per cent total solids all skim milks contained less than 0.01 per cent fat by the Babcock test. It was important to use milks of low fat content since fat retarded overrun development.

RESULTS

The data of tables 1 and 2 show that an increase in whipping tempera-

TABLE 1

The effect of temperature upon the whipping properties of dry skim milk reconstituted to 25 per cent solids

Whipping temperature	Overrun after whipping 3 min.	Temperature of room during tests	Stability of foam
°C.	*%*	*°C.*	*min.*
20	368	20	30
30	400	30	25
40	444	30	20
50	489	30	16
70	478	30	15

ture or a decrease in solids cause an increase in overrun but a decrease in foam stability.

TABLE 2

The effect of the concentration of solids upon the whipping properties of reconstituted dry skim milk

Solids content of mix	Overrun after whipping 3 min.	Stability of foam
%	%	*min.*
10	412	1½
15	422	5½
20	402	11
25	331	21
30	307	47

The experiments reported in table 3 show the improvement in whipping properties brought about by the high heat treatment of one skim milk while more detailed data on whipping and related tests with 9 dry skim milks are presented in table 4. Whipping data for 8 of these milks are plotted in figure 1.

TABLE 3

The effect of the heat treatment of a skim milk upon the whipping properties of its condensed or reconstituted dry product. All milks contained 30 per cent solids when whipped

No.	Treatment of skim milk sample	Overrun after whipping 3 min.	Stability of foam
		per cent	*min.*
1	Forewarmed at 65° C. 15 min., condensed to 30 per cent solids	106	0
2	Forewarmed at 95° C. 15 min., condensed to 30 per cent solids	240	7
3	Same as No. 2 but superheated to 100° C. after condensing	259	18
4	Same as No. 2, held cold overnight, heated to 50° C., spray dried	252	> 45

The results reported above were made the basis for experiments on some new food uses for skim milk. High foaming skim milks were whipped to stiffness at concentrations of 25 and 30 per cent solids and when sweetened, used as topping for beverages such as hot chocolate. These whips were used as a base for home frozen ice cream after the addition of flavoring and other fat-free ingredients. For food products requiring a permanent retention of air it was necessary to increase the stability of the skim milk foam. This was done by bringing about a mild coagulation of the casein after development of the whip, thus setting the foam structure. The use of heat, rennet, and acid as stabilizing agents was investigated. Attempts were made to start an incipient coagulation of the casein by heating the reconstituted milks over

TABLE 4

Whipping properties and heat stability of samples of reconstituted dry skim milk. The process used in the manufacture of these milks is given below fig. 1

No.	Overrun after whipping:			Stability of foam			Viscosity of drainage	Time of coagulation at 125° C.
	2 min.	5 min.	10 min.	2 min. whip	5 min. whip	10 min. whip		
	per cent	*per cent*	*per cent*	*min.*	*min.*	*min.*	*centipoises*	*min.*
1	371	436	484	55	56	54	43	17
2	310	400	443	107	120	90	87	60
2S	257	370	411	57	74	67	....	....
3	217	353	413	4	21	29	20	156
4	188	294	367	4	9	17	31	160
5	167	244	258	19	28	35	148	37
6	88	154	245	8	18	41	97	....
7	80	130	214		> 24 hours		....	15
8	29	93	180		> 24 hours		....	< 1

The whipped samples contained 30 per cent milk solids, the heat-coagulated milks contained 9 per cent solids, and sample No. 2S was No. 2 milk with 25 per cent milk solids, 15 per cent sugar, and 60 per cent water.

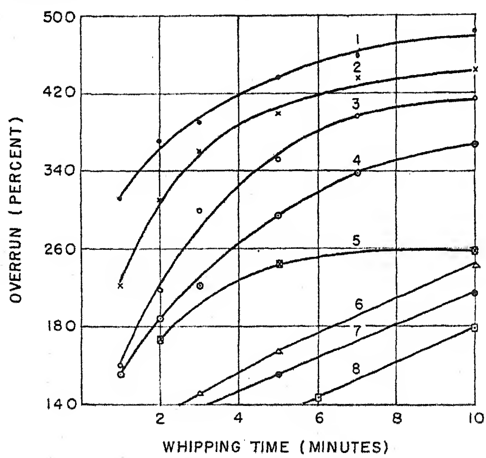

FIG. 1. Showing the development of overrun in dry skim milks reconstituted to 30 per cent solids and whipped for different periods of time. The dry skim milks were made by the methods indicated:

1. Vacuum drum dried commercial sample.
2. Spray dried commercial sample prepared for baking.
3. Fresh skim milk, forewarmed at 65° C., 20 minutes, condensed under vacuum to 30 per cent solids and spray dried.
4. Same milk and treatment as No. 3 but not condensed before drying.
5. Spray-dried commercial sample prepared for baking.
6. Same milk as No. 3, forewarmed at 95° C. for 20 minutes, spray dried.
7. Same milk and treatment as No. 3, but the condensed milk was superheated over a steam bath to 90–96° C. for 10 minutes and dried at once. Total time from start of superheating to end of drying operation was 45 minutes.
8. Atmospheric roll-dried commercial sample.

a steam bath while they were being whipped. The time required for the development of a coagulum was so long that the flavor was adversely affected and the method was not suited for stabilizing whips. The foam could be stabilized well with rennet when the factors controlling rennet coagulation were standardized, but this required exact laboratory methods. Nevertheless, a sweetened, rennet-stabilized, skim milk whip produced an attractive "Junket" type of desert when properly prepared. The foam was easily stabilized by the addition of acid. Pulped fruit from prunes, apricots, or berries was used as a source of acid. The product thus obtained made an excellent fruit whip which was equal to egg white fruit whips in flavor and often superior to them in stability. When pulp such as that of the prune was present, the whip was stable for many hours. Suitable formulas for fruit whips were developed. That for prune whip follows:

Dry skim milk	48 gr.
Powdered sugar	28 gr.
Water ($\frac{1}{2}$ cup)	113 gr.
Prune pulp	150–200 gr.

The skim milk and sugar were added to the water in the bowl of an electric mixer, the preparation whipped for at least 5 minutes and the prune pulp then quickly mixed in at low speed. Excessive mixing or stirring after the addition of acid foods caused some wheying off. Whipped mixtures of this type were frozen without stirring to produce smooth textured frozen desserts.

Fruit pulp was found to be necessary for the adequate stabilization of an acidified skim milk whip. Whips made with lemon juice wheyed off quickly, but when banana pulp and lemon juice were used, greater stability was attained. Excessive quantities of acid produced a whip with curd particles of objectionable size.

A milk powder flavor was sometimes noticeable in the finished whips. Fresh powder of good flavor was necessary for the production of an attractively flavored whip.

DISCUSSION

Foam of high air content and good stability may be secured by whipping properly heat-treated skim milks of 25 to 30 per cent solids content for several minutes. The data, however, are not complete enough to establish manufacturing procedures which will produce uniformly high whipping skim milks. The behavior of milks No. 3, No. 4, No. 6, and No. 7 (table 4 and fig. 1) indicates that increasingly severe heat treatment finally causes a decrease in whipping properties which is accompanied by a lowering in the heat stability of the protein. Milk dried on an atmospheric drum drier (No. 8) is subjected to intense heat when it is in a highly concentrated condition just before all the moisture is evaporated. The result of this drastic treatment is a powder of poor whipping properties and low heat stability.

It is probable that an optimum heat treatment which produces maximum whipping properties exists for each milk and that identical heat treatment does not necessarily produce the same whipping properties in different milks. Milk No. 4, table 3, developed 252 per cent overrun, while a milk obtained from the same source several weeks later and treated in the same way whipped to 367 per cent overrun with a stability of 83 minutes.

Commercial samples of dry skim milk prepared especially for baking whipped well, but some developed greater overrun than others. The preparation of a dry skim milk with high whipping properties should evidently involve high heat treatment somewhat similar to the procedure used in the manufacture of powder for baking purposes.

Particle size of insoluble roller dried powder exerts some influence upon air incorporation during whipping. An overrun of 156 per cent was produced after whipping a commercial powder of this type for 10 minutes. After the product was ground in a ball mill for 2 and 6 hours and whipped under the same conditions, overruns of 200 per cent and 251 per cent, respectively, were obtained.

SUMMARY

1. Reconstituted dry skim milks and condensed skim milks of 25 per cent to 30 per cent solids content were mechanically whipped in a few minutes to a stiff white foam. An overrun of 150 to 450 per cent and a foam stability of 10 to 90 minutes were found for different milks.

2. Wide variations were observed in the whipping properties of skim milks. High heat treatment usually improved whipping properties. Commercial milks prepared for baking purposes generally exhibited excellent whipping ability.

3. Skim-milk-whips were set by rennet or acid, but subsequent disturbance caused wheying off. Fruit-whips similar to an egg white product were prepared by adding sugar to the skim milk foam and stabilizing the whip by stirring fruit pulp into it.

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THE UTILIZATION OF UREA BY RUMINANTS AS INFLUENCED BY THE LEVEL OF PROTEIN IN THE RATION

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INTRODUCTION

The ability of ruminants to utilize simple nitrogen compounds such as urea or ammonium bicarbonate as a source of protein has been explained by bacterial synthetic activity occurring in the rumen and reticulum of these animals (1). A large and varied microflora is known to exist in the rumen and to account for this action. The media in which these microorganisms grow is determined by the ration fed. Since the kind and number of microorganisms are probably influenced by the composition of the medium, the possibility exists that by varying the ration fed, a change of the flora in the rumen could be produced and thereby a change in the synthetic reactions. In a previous "in vitro" experiment (2) it was found that the level of protein in a medium influenced the rate and amount of conversion of the ammonia (urea) when this medium was inoculated with microorganisms from the cow's rumen. As the protein level was increased from 2.5 grams to 5 grams of casein per 100 cc. of medium, the conversion of the added ammonia became negligible. The question arises as to whether this same phenomenon would occur in the rumen of animals fed urea. Acquisition of such knowledge would be important, not only from an academic viewpoint but also from a practical and economic one, since simple nitrogen compounds can be used as protein substitutes in rations of ruminants. With these facts in mind an experiment was set up to determine the effect of the level of protein fed on urea nitrogen utilization in the rumen.

EXPERIMENTAL

The data presented in this paper were obtained through the use of a 1000 pound Holstein heifer with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed twice daily at 8 A.M and 8 P.M. The daily ration consisted of corn silage 15 pounds, timothy hay 4 pounds, and a basal grain mixture of 4 pounds. The basal grain mixture was made up of ground yellow corn 50 per cent plus ground oats 50 per cent. The only variable in all the experiments was the grain mixture. These variations are listed in table 1. The heifer maintained its weight on

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the various rations fed throughout the experiment. Since the ration was always completely consumed within one hour after feeding, sampling was

TABLE 1

Variation of protein ($N \times 6.25$) content of the grain mixture

	Protein in basal concentrate theory	% protein increase due to L.O.M. added	% protein equiv. increase due to added urea	Total N as protein in concentrate	
				Theoretical	Actual
Experiment I—Variation of protein and urea levels					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	4	2	18.0	17.1
4	12.0	8	4	24.0	23.3
5	12.0	12	6	30.0	31.1
Experiment II—Variation of protein level with L.O.M.—urea constant					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	6	6	24.0	23.5
4	12.0	12	6	30.0	31.1
Experiment III—Variation of level of urea added—protein constant					
Trial 1	12.0	0	0	12.0	11.3
2	12.0	0	6	18.0	17.1
3	12.0	0	12	24.0	24.0
4	12.0	0	18	30.0	31.1

initiated at that time and continued throughout the day until the ammonia had returned to the basal level. All trials were repeated on alternate days at least three times before passing on to the next experiment. Table 2 contains the data obtained from three runs of the same trial as an example of the uniformity of results.

The determinations made on each rumen sample were ammonia, non-protein nitrogen (tungstic acid non-precipitable nitrogen), total nitrogen, and dry matter. The methods used in the sampling and in the determinations have been reported in a previous publication (3). With every ration tried it was found that the added urea-nitrogen was always hydrolyzed to ammonia within one hour after feeding. All results are calculated on a dry matter basis. When changing from one ration to another (change of the grain mixture composition) the animal was allowed to equilibrate for a week or more before sampling was started.

Utilization of the urea added was studied from three standpoints: first, varying both the oil meal and the urea added to the grain mixture; secondly, keeping the urea added constant and varying the oil meal; and thirdly, keeping the oil meal constant and varying the urea. In this manner the effect of both variables—urea and oil meal—on conversion could be studied. The results obtained are given in the accompanying charts.

TABLE 2

Data sheet experiment III, trial 3—analysis of rumen contents—urea added to basal grain mixture equivalent to 12 per cent protein

Hour after feeding	NH_3–N % Date				N.P.N. % Date				Total N (as protein) Date			
	12/15	12/17	12/19	Ave.	12/15	12/17	12/19	Ave.	12/15	12/17	12/19	Ave.
1	.317	.294	.276	.296	.522	.480	.470	.490	13.4	12.8	12.5	12.9
3	.192	.185	.206	.194	.348	.332	.322	.334	12.6	12.2	11.8	12.2
6	.044	.064	.066	.058	.222	.189	.183	.198	12.3	11.7	11.8	11.9
8	....	.033	.039	.036	....	....	....	....	....	....	....	....

DISCUSSION

In experiment I the concentrates consisted of mixtures having a protein content comparable to those sold commercially. Therefore it was desirable to know if urea would be utilized when these types of concentrates containing urea were fed with corn silage and timothy hay, a practical dairy ration. In figure 4 it is seen that all of the ammonia (urea) disappears at the end of six hours in all the trials but with one exception, namely, trial 5. In this trial the protein level of the grain mixture was 24 per cent and the urea added equivalent to 6 per cent of protein—a total of 30 per cent protein equivalent. This protein level in the grain mixture is one sometimes used, but is unnecessarily high. If the corresponding curves representing the total nitrogen

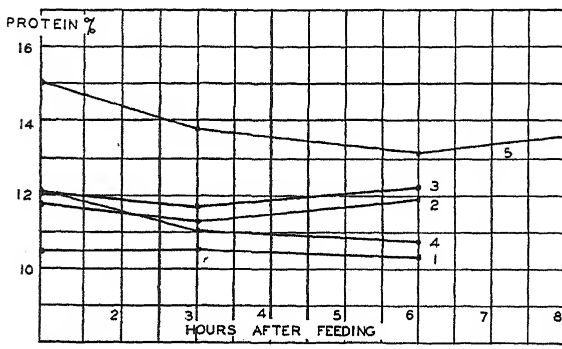

FIG. 1. Exp. I—Protein (N × 6.25) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

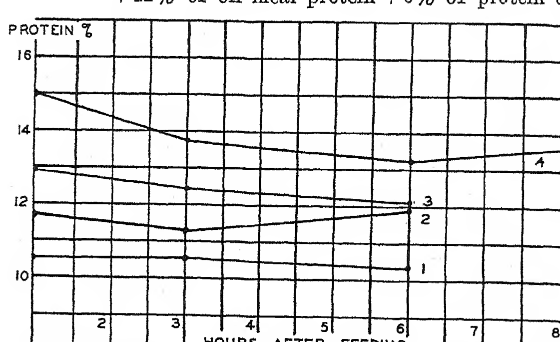

FIG. 2. Exp. II—Protein (N × 6.25) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

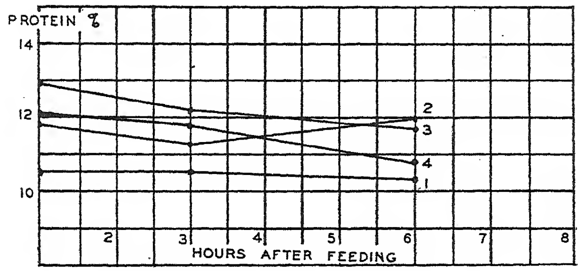

FIG. 3. Exp. III—Protein (N × 6.25) in rumen contents (dry basis).
1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " +12% " " " "
4. " " " " +18% " " " "

content of the rumen ingesta are examined (fig. 1) it is seen that here all the curves, again with one exception, lie quite close together (trial 5). In other words, excessively high protein levels found in the rumen will influence the rate of disappearance of ammonia. This verifies the results previously reported in "in vitro" experiments (2), where it was found that as the

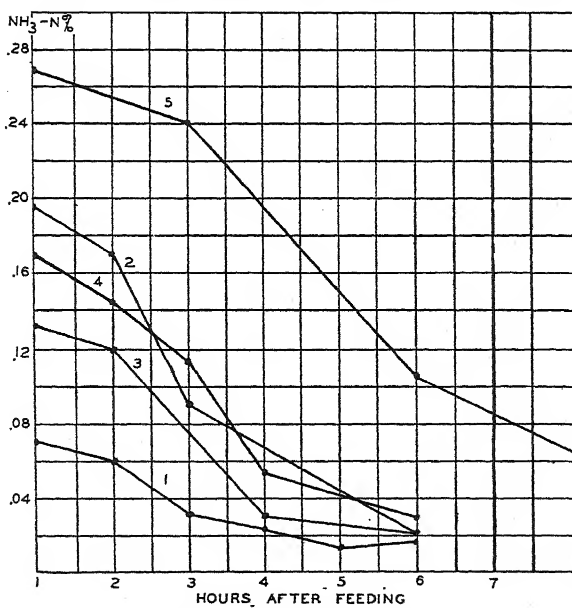

FIG. 4. Exp. I—NH_3-N in rumen contents (dry basis).
1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein +2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein +4% of protein equiv. as urea.
5. " " " " +12% of oil meal protein +6% of protein equiv. as urea.

protein (casein) in the medium was increased above 2.5 grams per 100 cc., the rate of conversion of inorganic nitrogen to protein was decreased. Judging from the results in experiment I, utilization of urea nitrogen will take place with protein concentrate levels as high as found in trial 4 (basal grain mixture plus oil meal to 20 per cent protein plus urea to 24 per cent protein equivalent) other parts of the ration being similar.

Examination of the results in experiment II (figs. 2 and 5, in which the urea added to the grain mixture is constant—6 per cent protein equivalent—and the protein content varied by adding oil meal) again illustrates the point that as the total nitrogen level in the rumen is increased above 12 per cent expressed as protein (fig. 2, trials 3 and 4) by feeding high protein concentrates (trial 3–18 per cent protein plus urea equivalent to 6 per cent protein; trial 4–24 per cent protein plus urea equivalent to 6 per cent protein) the utilization of ammonia is retarded (fig. 5, trails 3 and 4). Under the conditions of this experiment the utilization of urea fed at a level of 2.5 per cent in grain mixture decreased when the protein level of the concentrate was greater than 18 per cent.

When the low protein concentrate (grain mixture) equivalent to 11.3

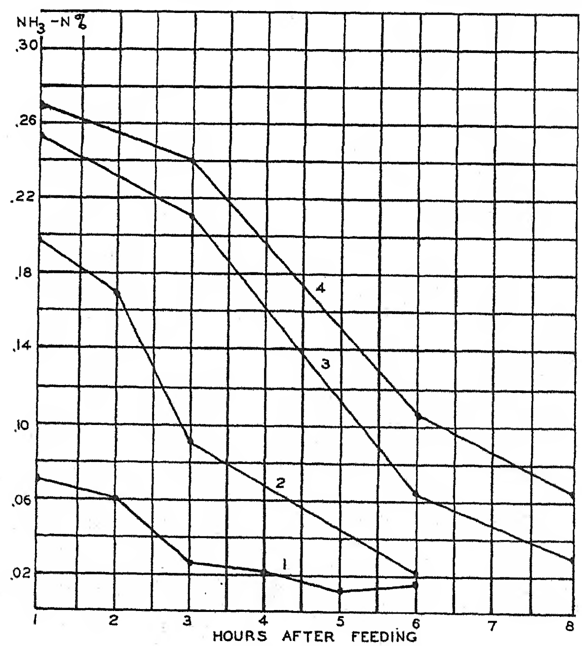

FIG. 5. Exp. II—NH_3-N in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

per cent protein was fed as in experiment III it was found that the urea added to the concentrate could be raised to a 4.5 per cent level (12 per cent protein equivalent) before there was a pronounced delay in the disappearance of the ammonia from the rumen (fig. 6, trials 3 and 4). It will also be noticed in figure 3 that the protein content of the rumen ingesta remained near the basal level. When large amounts of urea were added to this low protein concentrate as in trial 3 (12 per cent protein equivalent) and trial 4 (18 per cent protein equivalent) the rate of disappearance of the ammonia from the rumen was much faster (fig. 6) than when urea was added to the high protein concentrates as were fed in experiments I and II (figs. 4 and 5).

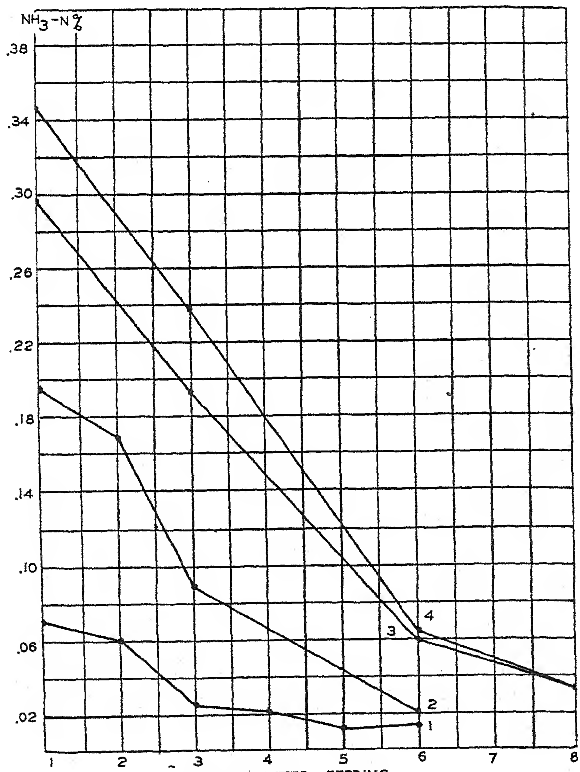

FIG. 6. Exp. III—NH_3–N rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " +12% " " " "
4. " " " " +18% " " " "

Figures 7, 8 and 9 contain the values obtained from determinations of non-protein nitrogen on several of the trials in each experiment. On

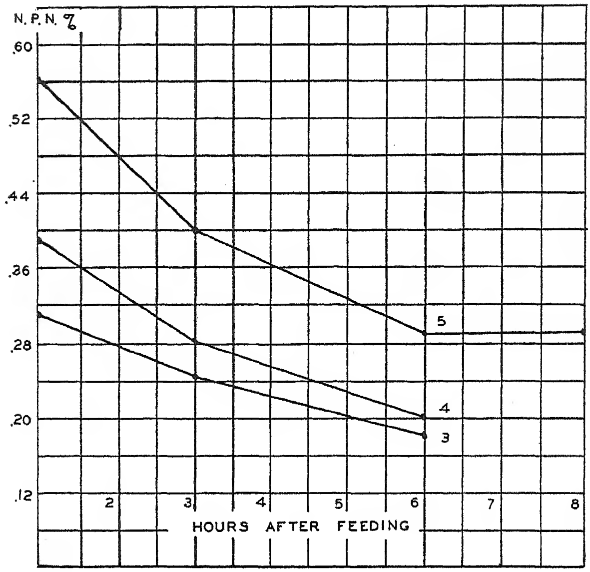

FIG. 7. Exp. I—N.P.N. rumen contents (dry basis).

3. Protein in Conc. 11.3% + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

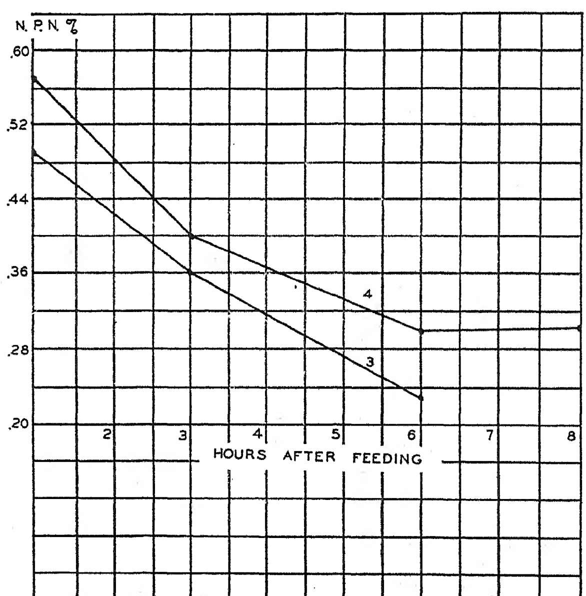

FIG. 8. Exp. II—N.P.N. rumen contents (dry basis).

3. Protein in Conc. 11.3% + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

examination it is seen that variations in these curves are directly related to the ammonia variation, since the ammonia is included in the non-protein nitrogen. When the variation in ammonia content is taken into consideration these curves become quite uniform.

The question may be raised as to whether the disappearance of ammonia is due to a conversion to protein by the microorganisms or simply a passage from the rumen unchanged. Some of the inorganic nitrogen may leave the rumen without being converted to protein. However, in view of the present experiments where it was possible to show a decided delay beyond 6 hours in the decrease of ammonia by a high protein content of the rumen ingesta

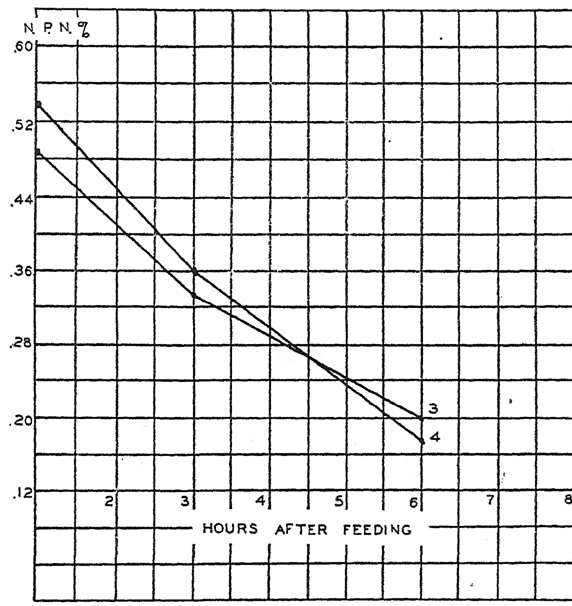

FIG. 9. Exp. III—N.P.N. rumen contents (dry basis).
3. Protein in Conc. 11.3% + 12% protein equiv. as urea.
4. " " " " + 18% " " " "

it appears that the disappearance taking place must also be due to a conversion to protein. Also, evidence of protein formation from urea in the rumen has already been reported in a previous publication (3).

Another factor which might easily influence the rate and extent of conversion of ammonia to protein in the rumen is the available carbohydrate fed. Experiments are now in progress to determine how the amount and kind of carbohydrate and the length of time it remains in the rumen will affect the rate of utilization of ammonia.

SUMMARY

1. The protein content of rumen ingesta showed a decided increase when the level of protein in the concentrate fed was increased to 24 per cent.

2. The rate of conversion of urea nitrogen to protein in the rumen decreased as the protein level of the rumen ingesta became greater than 12 per cent.

3. When the level of protein in the concentrate fed was increased to more than 18 per cent the rate and extent of conversion of added urea nitrogen to protein began to decrease.

4. When no linseed oil meal was added to the basal grain mixture (11.3 per cent protein), the added urea was utilized up to a level of 4.5 per cent (protein equivalent of 12 per cent) of the grain mixture.

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EFFECT OF STILBESTROL ON THE MAMMARY GLAND OF THE MOUSE, RAT, RABBIT, AND GOAT*

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If knowledge of the hormonal causes of the growth and lactation of the mammary gland is to be put to practical use it will be necessary to find cheap sources of the hormones and devise simple and effective methods of administration. This report is concerned with a first step in that direction. Work in this laboratory has shown that the estrogenic hormones stimulate growth of the mammary duct system by increasing the rate of secretion of mammogen by the pituitary (1).

The recent synthesis of a compound called stilbestrol, which has very great estrogen-like physiological activity and still is relatively inexpensive in comparison to the natural estrogens, has stimulated considerable research. Stilbestrol has the further advantages that it is quite effective orally and when formed into pellets is slowly absorbed for months when implanted under the skin (2, 3, 4, 5). This chemical, which simulates the estrogens in its physiological properties, is produced at 1/50 the cost for equal activity. It is sufficiently low in cost at active dosages to consider its practical application in livestock therapy. With this in mind the authors are conducting experiments on the influence of stilbestrol on the mammary gland and milk secretion in laboratory and farm animals.

Many stilbestrol experiments of clinical nature or having no application to mammary development cannot be reviewed here (for these see ref. 6). Assays of the effect of stilbestrol on the genital organs show that one-half gamma produced estrus in 70 per cent of 175 gram spayed rats (7). Stilbestrol was found to be equal to, or five times as active as, estrone in producing vaginal cornification in rats and mice (4, 7, 8). Orally administered stilbestrol was twenty times as active in mice as estrone and 16 times as active as estradiol (7).

Van Heuverswyn *et al.* (9) found that groups of five male mice given 0.05, 0.2, and 2.0 mg. of stilbestrol, respectively, gave plus two, plus three and plus one rating of mammary duct responses after eight injections in 16 days.

Stilbestrol was found to be about one-fifth as active as estrone in causing proliferation of the mammary gland of spayed rats (10), and of guinea pigs (11).

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In contrast to these results, experiments conducted by the present authors showed stilbestrol to be more active than estradiol benzoate in causing mammary duct proliferation in male mice (12).

Mammary development in the human male from oral administration of stilbestrol has been reported (13). A total of 480 mg. were given over 96 days. Hard, firm masses 6 cm. in diameter and 2.5 cm. thick were palpable under the nipples. These masses were harder, and denser than result from the action of the natural estrogens. Similar results, although less development, were obtained in a six-year-old girl. Breast hypertrophy and genital changes were reported (14) in a castrate woman given 20.25 mg. of stilbestrol in 18 days followed by 30 rabbit units of progesterone in six days. MacBryde *et al.* (15) reported similar results with stilbestrol administered orally or by injection.

Proliferation of the mammary epithelium was revealed by biopsy specimens obtained before and after oral administration of 280 mg. of stilbestrol to a castrate woman. One milligram a day given orally for 14 days to a second woman caused painful breast swelling (16).

De Fremery (17) reported in 1936 that inunction of the udders of virgin female goats with estradiol benzoate caused mammary growth. Initially slow udder development increased abruptly with changes of parturient type. Lactogen then produced abundant normal lactation.

Folley *et al.* (18) reported that stilbestrol dipropionate in oil applied to the udders of three virgin female goats, plus daily milking, caused the production of a maximum of 1500 cc. of normal milk daily. A normal lactation curve resulted. A virgin heifer similarly treated responded with colostrum secretion alone. Attempts to induce lactation with stilbestrol in male goats failed even when progesterone was added to the treatment.

Lewis and Turner (19) reported that daily subcutaneous injections of stilbestrol caused the initiation of lactation with a maximum production of about half the normal quantity of milk from two yearling goats during lactation periods of six months. A castrate and a normal kid lactated for 95 days. The effect of stilbestrol injections on two goats already in milk was not good as far as production was concerned.

PROCEDURE

Stilbestrol (4:4 dihydroxy α β diethyl-stilbestrol) was obtained in powder form from E. R. Squibb and Sons. For injection it was dissolved in ether, added to the oil carrier and the ether removed before a fan. The daily dosage was administered in 0.05 to 0.2 cc. of oil to mice and rats and 0.1 to 0.4 cc. to rabbits. Two milligram tablets of stilbestrol, from the Geo. A. Breon Co., Kansas City, were put in suspension in the drinking water for oral administration to mice and renewed daily.

The mammary glands were removed in toto at biopsy or necropsy, fixed

in Bouin's fluid and stained in Mayer's hematoxylin. After removal of the paniculus carnosus muscle and overlying connective tissue, the glands of the rabbits were measured and the greatest diameter recorded. Rabbit glands were removed at about 20-day intervals. Pituitary lactogenic hormone was prepared by the Bergman-Turner (20) method in this laboratory from cattle anterior pituitary and was assayed by the McShan-Turner pigeon method (21). One international unit equals 0.8 McShan-Turner units. Three hundred and forty international units (approximately 11 i.u. per 100 gm. body weight) are required to produce lactation in normal pseudo-pregnant rabbits and constitute one rabbit unit (22). It was given to rabbits in 6 equal daily subcutaneous doses. The mammary glands were examined and a gland removed on the seventh day.

Goats were injected subcutaneously in the crops with stilbestrol in 0.2–0.5 cc. of oil daily. Half of the udder was removed under local anesthetic, frozen, cross sectioned by hand, fixed in Bouin's fluid, stained and mounted in isobutyl methacryolate polymer. Thinner, small sections were mounted on slides in clarite. Paraffin sections were also made.

The mice were fed a mixed grain ration containing cod liver oil plus Purina dog pellets. The rabbits were fed a mixed grain ration plus alfalfa hay. Goats were stabled continually and fed a mixed dairy ration plus alfalfa hay.

EXPERIMENTAL RESULTS

Mice. Groups of three to five male mice injected with stilbestrol daily for 14, 21, and 27 days responded with extensive development of the mammary duct system (table 1). Dosages of 0.167 to 0.5 gamma per day caused the production of glands extending 0.5 to 1.5 cm. in length, usually with fewer main ducts than in glands of normal female mice. In most cases early interlobular duct development was evident. In one case isolated clumps of secreting alveoli were found, as reported by Gomez and Turner (23) following anol treatment (fig. 1). This condition has been shown in this laboratory (unpublished) to result from estrone injection. In no case were lobules of alveoli well developed. There was considerable variation in development of glands from the same mouse, but large glands were found in each case. There was progressive development in the groups treated for 14, 21, and 27 days.

Groups of four spayed virgin female mice given 0.167, 0.5 and 1.5 gamma of stilbestrol per day for 11 to 21 days gave a varying response. In some cases only end-buds and duct growth were apparent, in others interlobular ducts had developed. A number had mammary elements expanded with secretion. Two showed small, isolated clumps of alveoli (fig. 1).

Stilbestrol has been reported to be comparatively much more active orally than the natural estrogens (8). Oral administration would appear to be of considerable advantage in practical application of hormone therapy

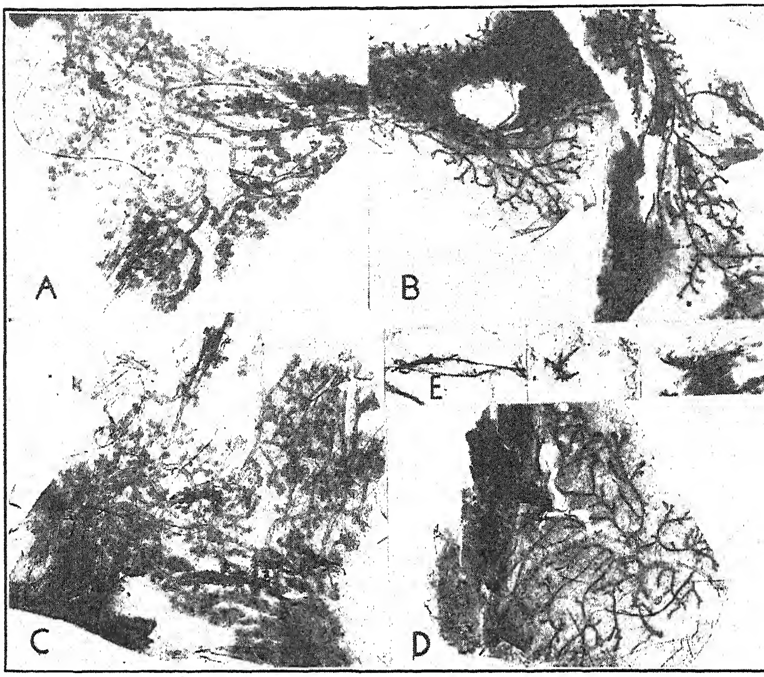

FIG. 1. a. Mammary gland from a spayed virgin mouse after daily injection of 1/6 γ of stilbestrol for 20 days. The ducts were expanded with secretion as were clumps of what appear to be alveoli. $\times$ 4.8.

b. Control mammary glands from virgin female mice. Notice the smooth, unexpanded ducts. $\times$ 1.5.

c. Mammary gland from a male albino mouse after 21 days treatment with 1/6 γ of stilbestrol daily. This gland was developed similarly to those of the female mouse shown in (a). Only one such case was found among a large number of males treated with stilbestrol. $\times$ 4.8.

d. Typical mammary gland from a male mouse treated with stilbestrol for 14 to 27 days. Note active hyperplasia of ducts shown by enlarged, deeply staining end-buds. Some of these mice had ducts made rough in appearance by the beginning of interlobular duct development. This gland was similar to control glands in (e) before treatment. $\times$ 4.8.

e. Three typical control mammary rudiments from male mice. $\times$ 4.6.

to livestock. Stilbestrol administered in the drinking water to male mice caused extensive duct proliferation similar to that caused by injection. Groups of three and four mice were treated for 14 and 21 days. One-tenth to 0.4 gamma per day gave little or no stimulation while 0.5 to 1.5 gamma per day gave extensive proliferation (table 1).

From these results it is seen that stilbestrol readily caused extensive duct development in male mice either by injection or when administered in the drinking water. Stilbestrol carried the stage of gland development

TABLE 1

Effect of stilbestrol on the mammary gland of the mouse

Mode of adminstration	Number of mice	Days treated	Dosage, gamma per day	Results
Subcutaneous injection, males	5	14	1/6	1 neg. 4 pos. Largest gland in each case 0.6 to 0.9 cm.
	5	21	1/6	5 pos. Most glands 0.5 to 1 cm. diameter. One mouse had isolated alveolar clumps.
	4	27	1/6	Most glands 0.7 to 1.6 cm. extent. Roughened ducts.
Subcutaneous injection, males	5	14	0.5	Glands +1 to .9 cm. Largest .5 to .9 cm. in each case.
	5	21	0.5	Most glands .4 to .9 cm. extent. Roughened ducts.
	5	27	0.5	1 barely positive. Others, most glands 0.5 to 2 cm. extent. Roughened ducts.
Oral, in drinking water	3	14	0.1	One with teats had .4 to .6 cm. glands. Others negative.
	3	21	0.1 0.2 last 7 days	Negative.
	3	14	0.2	One with +1 gland. Others negative.
	3	21	0.2 0.4 last 7 days	Negative.
	3	14	0.4	One with a +1 gland. Others negative.
	3	21	0.4 0.8 last 7 days	2 strongly developed with .5 to .9 cm. ducts.
Males	3	14	0.5	Largest glands 0.6 to .8 cm.
	4	21	0.5	Most glands 0.4 to 1.2 cm. ducts.
	4	14	1.0	0.5 to 1.1 cm. roughened ducts.
	4	21	1.0	0.5 to 1.5 cm.
	4	14	1.5	0.5 to 1.1 cm. roughened ducts.
	4	21	1.5	0.5 to 1.0 cm. roughened ducts.
Injected, spayed females	4	11	1/6	1 with end-buds, 2 interlobular ducts.
	4	20	1/6	2 with clumps of alveoli. 2 with roughened ducts.
	4	14	0.5	2 with end-buds. No lobules. 2 roughened ducts.
	4	21	0.5	Ducts thickened with secretion. Interlobular ducts—No lobules.
	4	14	1.5	Roughened ducts. Glands rather small in 2 cases. End-buds 1 case.
	4	21	1.5	Thick roughened ducts—2 cases. End-buds—1 case. No lobules.

merely to that obtainable with the natural estrogens in the mouse. No true lobule development occurred, such as is found in pregnancy, even in spayed virgin females.

A total dosage of 0.05 γ in six days was reported to cause a mammogenic duct growth mouse unit response (12). In this study 0.167 to 0.5 γ per

day caused extensive duct development. Oral administration appeared to require about six times as much hormone as by injection.

Rats. Subcutaneous injection of stilbestrol into groups of three castrate virgin female rats at 0.004, 0.008, 0.017 and 0.034 gamma per day did not result in any obvious signs of mammary duct growth. Single glands were removed on the 7th, 14th, and 23rd days of treatment. The remaining glands were recovered and examined in toto after sacrificing on the 28th day (table 2). The mammary glands appeared to be in active rather than in regressed condition in most cases, however, staining deeply. Several glands removed had main ducts which were considerably thickened by side development of interlobular ducts and perhaps alveolar buds. No duct end-buds were apparent.

Three groups of similar rats given 0.25, 0.5 and 1.0 gamma per day of stilbestrol for 18 days all showed active proliferation of mammary ducts.

TABLE 2

Effect of stilbestrol on the mammary gland of the rat

Number of rats	Av. weight after treatment	Dosage, gamma per day	Results			
			7 days	14	23	28
	gm.					
3	177	0.004	Glands were all negative for additional duct growth.			
3	169	0.008				
3	191	0.017				
3	169	0.034				
			18 days			
3	111	0.25	Many duct end-buds and considerable proliferation of ducts.			
3	111	0.50				
2	106	1.00				

In this study four times the mouse unit dosage of stilbestrol gave no demonstrable signs of duct proliferation. Duct growth was obtained with a dosage of 0.25 γ per day which is at least 30 male mouse units (12). However, the minimum dosage to secure duct growth in the rat was not ascertained. This is in contrast to results secured with the natural estrogens in which the rat mammary gland appeared to respond to lower dosages than did that of the mouse (24, 25).

Rabbits. Twelve rabbits either injected or implanted with stilbestrol showed extensive mammary proliferation. Glands removed from males after injection of 4 to 32 gamma per day for 20 days had a complex duct system with an average extent of about 3 cm. (tables 3 and 4). This was not extensive development but glands from rabbits given 4 γ were as large as those from rabbits given 32 γ a day, indicating that 4 γ were adequate to secure the maximum rate of development. Since it appeared that the dosage might still be above the optimum, the dosage to four of the rabbits was then reduced to 1/10 that of the first 20-day period (0.4 γ–3.2 γ) and

single glands were removed by biopsy at 40 and 60 days. The 40-day glands averaged 4.4 cm. in extent and the 60-day glands 5 cm. The rabbits were sacrificed at 70 to 80 days of injection when all remaining glands averaged over 6 cm. They compared favorably in size with those of virgin female rabbits but were in several cases more complex. These glands consisted not only of duct systems but in addition had considerable lobule development (fig. 2).

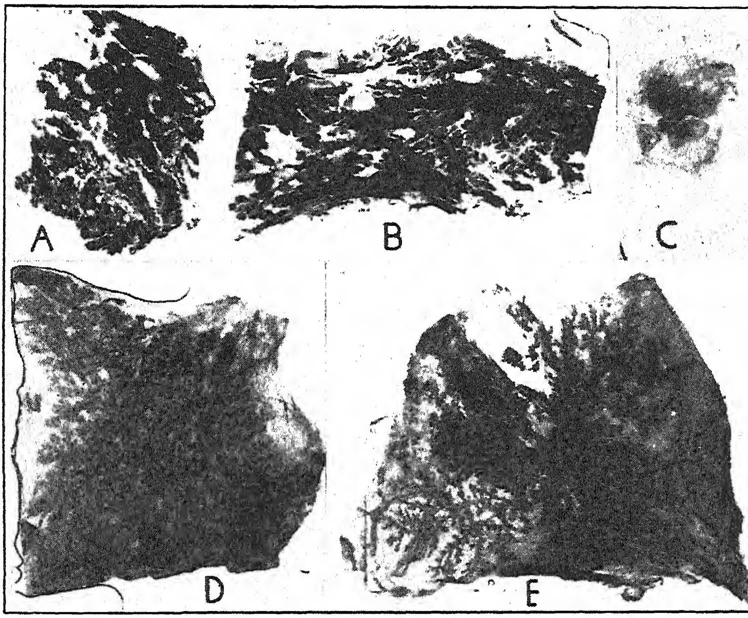

FIG. 2. a. Section of mammary gland from a virgin female rabbit (No. 24) given 32 γ daily of stilbestrol for 20 days. This gland contained copious quantities of milk when removed. There was a well developed lobule system with hypertrophied alveoli. $\times 1$.

b. Section of a mammary gland from a male rabbit (No. 29) with an 18.7 mg. pellet of stilbestrol subcutaneously. This gland was obtained 97 days from implantation of the pellet. The extensive lobule-alveolar system was expanded with milk from administration of a rabbit unit of lactogen. Compare with control gland (c). $\times 1$.

c. A typical control gland from a male rabbit. $\times 1$.

d. Half of a mammary gland from a male rabbit (No. 22) given 16 γ of stilbestrol daily for 20 days and then 1.6 γ for 45 days. Interlobular ducts are well developed and a few areas appear to have small lobules with alveoli. $\times 1$.

e. Mammary gland from a male rabbit (No. 14) given 32 γ of stilbestrol daily for 20 days and then 3.2 γ for 50 days. Lobules have developed in the center of the gland. $\times 1$.

An attempt was made to secure milk secretion from the small duct systems of four of the male rabbits after 20 days treatment with stilbestrol.

TABLE 3

Response of rabbit mammary glands to stilbestrol and lactogen

Sex	Mode stilb. admin.	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 27-day glands	Type of development	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 54-day glands
			Dosage 6 days	Response				Dosage 6 days	Response	
					cm.					*cm.*
Male 5	Injection	32 γ	10 i.u./100 gm. body weight	Serous	2.7, 3.4	Interlobular ducts				
" 14	"	32 γ		Serous	2.8	Early lobules				
" 4	"	16 γ		Serous	3.5	Ducts				
" 3	"	8 γ		Milk	2.7	Lobules	8 γ	10 i.u./100 gm. body weight	Copious milk	5
" 13	"	4 γ		Serous	3.8	Ducts				

Lactogen was given at the rate of 10 international units per 100 gm. body weight in six daily injections. At operation for removal of sample glands, those of three rabbits showed serous secretion and expanded ducts but no milk. Milk could be squeezed from the teats of the fourth rabbit and the small duct system was filled with milk. After 20 more days treatment with stilbestrol this rabbit had a 5 cm. duct system which again filled with milk on lactogenic treatment.

Three 1200 to 2500 gram virgin female rabbits injected with 8, 16, and 32 γ daily of stilbestrol had 5 to 7 cm. glands removed after 20 days of injection. The gland from the rabbit on 32 γ was engorged with milk in a lobule-alveolar system. The glands from the other two rabbits had no milk but the main and interlobular ducts present were swollen with serous secretion.

Glands removed at 40 days of injection did not show such obvious secretion. In two cases interlobular ducts were present while in the third early lobules were present. The glands removed after 60–65 days had in two cases unhypertrophied lobules. In the third only interlobular ducts were present.

Two male rabbits implanted with 16.8 and 18.7 mg. pellets of stilbestrol showed no mammary development in glands removed at 20 days after implantation (table 4). The 40-day glands removed were 4 to 5 cm. in extent, however, and showed what was apparently the beginning of lobules, perhaps consisting of intralobular ducts. Short central ducts were highly cystic. At 60 days the lobules were more apparent. Glands removed at 80 days appeared to have true lobules with alveoli. These were rather scattered and small and did not constitute the pseudo-pregnant gland condition. These glands were approximately 6 cm. in extent. At 90 days lactogen was administered at the rate of 12 i.u. per 100 grams body weight. The mammary glands became swollen with milk so that it could be expressed from the teats. Glands removed on the 97th day were composed of hypertrophied lobules of alveoli and ducts full of milk (fig. 2). Three glands removed from one rabbit at necropsy at 103 days averaged 9.5 cm. in extent. The second rabbit was sacrificed at 122 days when two 10 cm. lobule-alveolar glands were removed. These glands contained isolated areas composed of apparently abnormal lobules with very large alveoli, as seen by Gomez and Turner (23) after anol treatment of rabbits.

Five rabbits given 0.02 γ to 2.0 γ per day per teat applied in alcohol to the shaved skin for 30 days responded with growth of the mammary glands. Two of these rabbits which received 2 γ and 0.2 γ per teat had glands averaging 4.2 cm. and 4.7 cm. in extent. Some of these glands were over 5 cm. in diameter. Development had progressed to the interlobular duct or early lobule stages. The teats were also considerably enlarged over the control condition. This also occurred in the rabbits injected with stilbestrol and in those with pellets.

TABLE 4

Response of rabbit mammary glands to stilbestrol and lactogen

Sex		Mode stilb. admin.	Dosage stilb. /day	Dia. 20-day glands	Type of development	Dosage stilb.	Dia. 40–43 day glands	Type of development	Dia. 60–65 day glands	Type of development	Dia. 70–80 day glands	Type of development
				cm.		*gamma*	*cm.*		*cm.*		*cm.*	
Male	14*	Injection	32 γ	2.3	Lobules	3.2	4.0	Lobules	2.4	Lobules	5.6, 5.7	Lobules
"	22	"	16 γ	3.2	Ducts	1.6	5.7	Interlobular ducts	5.5	Interlobular ducts	5.7, 6.1	Interlobular ducts
"	23	"	8 γ	2.5	Ducts	0.8	4.0	Ducts	4.4 5.7	Plain ducts Interlobular ducts	6.6, 7.7	Early lobules
"	13*	"	4 γ	3.5	Ducts	0.4	4.0	Early lobules	5.6	Ducts	6.4, 5.0	Interlobular ducts
Virgin female	24	"	32 γ	6.6	Lobules Alveoli small Milk in ducts		5.7	Lobules of alveoli	8.2 7.0 9.0 10.0	Lobules		
" "	25	"	16 γ	5.0	Ducts Serous secretion		6.0	Lobules	8.0	Lobules		
" "	26	"	8 γ	5.0	Interlobular ducts Serous secretion		5.5	Swollen ducts	8.5 6.7	Ducts		
Male	28	Subcutaneous pellets	16.8 mg.	Neg.			5.0	Early lobules	4.6	Lobules	6.0	Lobules
"	29	Subcutaneous pellets	18.7 "	Neg.			3.9	Early lobules	4.3	Early lobules	5.5	Early lobules

	Lactogen treatment at 90 days		Dia. 97 day glands	Type of development	Dia. 103 day glands	Type of development	Dia. 122 day glands	Type of development
	Dosage	Respone						
			cm.		*cm.*		*cm.*	
Male 28 (continued)	12 i.u./100 gm. body weight	Copious milk	6	Cystic ducts Large lobules	8.8, 10.2	Lobules Small alveoli		
" 29 (continued)		Copious milk	12	Isolated lobules, hypertrophied	9.5		9.8 5.8, 10.0	Small alveoli Lobules

* Days elapsed between the two series of injections, rabbit #14–26 days; rabbit #13–40 days.

Male rabbits responded with extensive mammary development on injection of as little as 0.4 γ per day of stilbestrol or on percutaneous application to the shaved skin. It is interesting to note that in the male rabbit the lobule-alveolar system developed to a greater extent than in the female mouse similarly treated. Alveolar lobules began to appear after 20 to 40 days treatment in the rabbit and were well developed in rabbits implanted with pellets of stilbestrol.

Well developed mammary duct systems grown with stilbestrol in male rabbits came into lactation upon administration of lactogen. It was not necessary to terminate the stilbestrol treatment during lactogen administration for male rabbits with subcutaneously implanted pellets responded readily. Stilbestrol was thus shown to have the estrogenic property of preparing the mammary gland to respond to lactogen by the production of milk (26, for review).

A normal adult female rabbit did not require the administration of lactogen, for it responded with copious milk secretion to stilbestrol injection alone. This has also been shown to occur in the dry female goat (18, 19). Stilbestrol appears to cause the release of lactogen from the animal's own anterior pituitary in these cases, for a goat brought into milk with stilbestrol had at least twice the lactogen content in her urine as did normal dry goats (19).

Goats. Six goats were given subcutaneous injections of stilbestrol for periods from 96 days to 8 months. The substantial lactations which re-

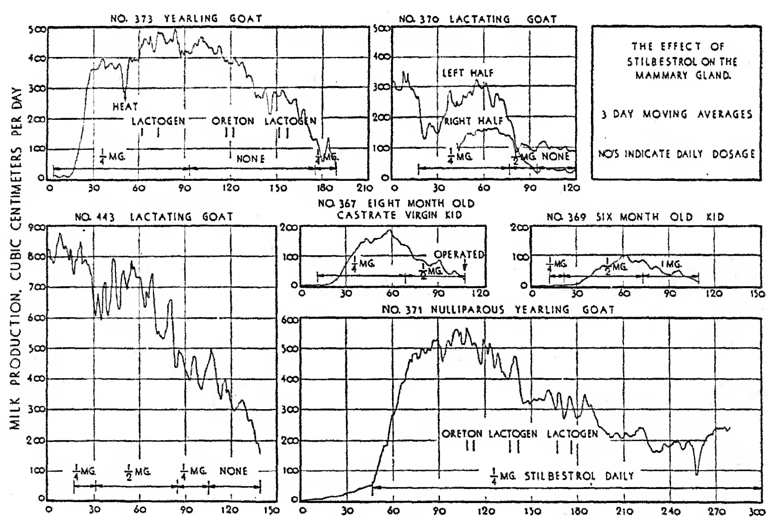

FIG. 3. The effect of daily, subcutaneous stilbestrol injection upon lactation in a castrate and a normal virgin kid, two yearlings goats which had never lactated and two mature goats in milk.

sulted from two kids and two yearlings has been previously reported as was the effect on the milk production of two goats which were already in lactation before the beginning of treatment (19). These lactations have been charted and are reproduced here (fig. 3).

The two virgin female kids received daily injections of stilbestrol for 96 days. The kids were six (No. 369) and eight (No. 367) months old when treatment was begun in July, so presumably they had had no estrous cycles. Goat No. 367 was castrated shortly before stilbestrol treatment was begun. Initial dosage was 0.25 mg. per day raised to 0.5 mg. on the 58th day; a total of 34.25 mg. in 96 days. Goat No. 369 was given 0.25 mg. per day for 12 days, 0.5 mg. for 44, 1.0 mg. a day for 41 days; a total of 67 mg. in 96 days.

At this time half of the udder was removed from each kid. The mammary glands were found to be about 4 cm. in diameter and consisted of ducts with thick clusters of lobules as in normal lactating glands after parturition, although most of the alveoli were no longer secreting heavily. Occasional alveoli or even lobules appeared to be still quite active (fig. 4). The lobules from the normal kid (No. 369) appeared to be more compact and fully developed than those from the castrate (No. 367). The extent of the glands does not appear to have been much increased but the lactation induced had caused the glands to expand into spherical form and had developed the gland cisterns. A mammary gland removed from a 37 day pregnant goat did not have nearly as complete a development of the lobules. Another gland removed from a 74 day pregnant kid appeared to have a complete lobule-alveolar system which was unhypertrophied, however (fig. 4). The alveoli were small masses of cells without lumina. This gland measured 0.5 cm. in thickness and 5.5 by 3.5 cm. in extent.

In contrast to the glands from stilbestrol treated kids Turner and Gomez (27) have shown that the mammary gland of the immature female goat consists of a thin, one centimeter or less, layer of ducts lying at the base of the teat and extending a few centimeters from it. Gland sections showed that the mammary system although rather complex consisted of a two cell layered duct system without lobules. Short, wide branches of the smaller ducts which gave the impression of alveolar buds were also two layered except the distal ends which were composed of solid masses of epithelial mammary cells.

A mammary gland removed from a mature male castrate goat (No. 833) showed no development after extensive stilbestrol treatment (fig. 4). For 78 days 0.25 mg. of stilbestrol was given daily by injection, a total of 19.5 mg. The teats were then enlarged considerably. Twice daily milking was instituted on the 52nd day but the yield was only 0.5 to 1 cc. per day for 10 days. No treatment was given from the 79th to the 118th day. Then three small hard pellets of stilbestrol, a total of 71.75 mg., were implanted sub-

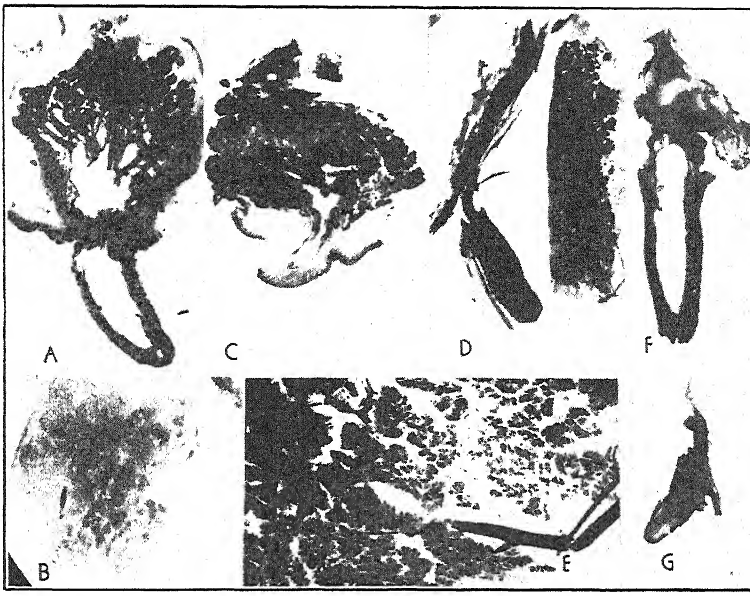

Fig. 4. a. Mammary gland section from castrate virgin kid 367 after 96 days treatment with stilbestrol. There is a well developed lobule alveolar system. The teat is enlarged. ×.64.

b. Enlarged microsection from mammary gland of goat 367 showing hypertrophied alveoli. ×3.

c. Mammary gland section from virgin kid 369 after 96 days treatment with stilbestrol. This gland is similar to that from goat 367 except that it appears more compact partially because no fluid was injected into the gland during fixation. The teat section here is incomplete. The teat from this gland was equal in size to that of goat 367. ×.64.

d. Dorso-ventral section with teat from gland of a 74 day pregnant kid and a lateral section from the same gland. This gland was flat compared with the globular shape of glands from goats 367 and 369. ×.64.

e. Enlarged section from gland of 74 day pregnant kid showing lobules. The alveoli in this gland were unhypertrophied clusters of cells. ×3.

f. Mammary gland and teat from a mature castrate male goat after 78 days injection of stilbestrol and 75 days with 71.75 mg. of subcutaneous pellets. The teat is obviously enlarged but the gland consisted of a short duct and a cluster of mammary cells at the base of the teat. ×.64.

g. Section of teat and mammary gland from an untreated castrate male goat. Compare size of teat with that in (f). The mammary gland consists of two very small clusters of cells at the base of the teat. ×.64.

cutaneously. A mammary gland was removed on the 292nd day which consisted of a teat cistern and a small clump of mammary cells a few millimeters in diameter. The absorption rate of 100 mg. stilbestrol pellets in women was found to be 0.127 to 0.25 mg. per day and they were effective for 400 to 800 days. Pellets were reported to be 5 to 10 times as effective

as injection on a dosage basis (5). Proportionally 0.09 to 0.18 mg. of stilbestrol should have been available to cause mammary development in this male goat but none occurred. The teat had developed, however, for it measured 4.5 cm. in length and was more than twice the length of that found in control male goats.

Turner and Gomez (27) found that the mammary gland of a six-months-old male goat was only one centimeter in diameter. That from a four-year-old male was 5 cm. in extent. The duct system extended little beyond the base of the teat, however. In exceptional males the glands may attain considerable development and may even lactate. A gland removed in this study from a ten-months-old male goat castrated at 8 months of age consisted of a teat cistern and a very small cistern at the base of the teat (fig. 4). This cistern was surrounded by several layers of epithelial mammary cells the extent of which was under 0.5 cm. The teat was two centimeters in length.

This study has shown that stilbestrol caused little duct development but extensive lobule-alveolar hyperplasia in a castrate and a normal kid treated subcutaneously for 96 days. Abundant and prolonged lactation occurred. Extensive stilbestrol treatment both by injection and with pellets in a mature castrate male goat failed to cause mammary development. Only the teats developed as did those of the kids treated.

DISCUSSION

Stilbestrol proved to be a very active mammary duct growth factor in mice, rats and rabbits. It was rather surprising to find that in female goats treated for 96 days instead of extensive duct growth there had occurred the proliferation of rather restricted lobule-alveolar systems. Early lobule development was found in the injected male rabbits and more extensive development in those with pellets but this only occurred after extensive duct growth. The ovaries could hardly have been a primary factor in the lobule development in goats, for one of the kids had been castrated before initiation of treatment. For the same reason the condition of the ovaries was probably not instrumental in initiating, through action on the pituitary, the lactation which occurred in these and the yearling goats. In fact the castrate kid responded more readily than the normal one and produced a greater amount of milk.

It remains to be seen whether extensive duct growth can be obtained in the goat with stilbestrol. In laboratory animals the male responds readily to estrogen treatment with proliferation of mammary ducts. The failure with stilbestrol in the male goat treated may have been due to inadequate dosage.

SUMMARY

Subcutaneous injections of stilbestrol at low dosages caused extensive

duct proliferation in male mice in 2 to 4 weeks. Mammary development did not proceed farther in spayed virgin female mice similarly treated. Oral administration of stilbestrol to male mice required approximately six times as high a dosage as by injection to obtain similar results.

Castrate male rats required a higher dosage of stilbestrol than did mice to obtain mammary duct growth.

Four-tenths gamma per day of stilbestrol subcutaneously was adequate to secure extensive mammary duct development in male rabbits. After 40 to 60 days treatment early lobule development was apparent. Percutaneous administration was also effective. Mammary glands from two rabbits with subcutaneous pellets had well developed lobule-alveolar systems and responded well to lactogen treatment at 90 days. Normal females tended to lactate on stilbestrol injection alone.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from lobule-alveolar glands. Little increase in extent of glands was apparent. Subcutaneous administration followed by pellet implantation caused no mammary gland development in a castrate male goat, although the teats were hypertrophied.

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THE CONTENT OF GRASS–JUICE FACTOR IN LEGUME SILAGES AND IN MILK PRODUCED THEREFROM*

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In previous publications (1, 2, 5) from this laboratory it has been shown that many plant materials contain an unidentified water-soluble growth-promoting substance for rats and guinea pigs. Because of its abundance in young grass this substance has been called "grass-juice factor." Johnson *et al.* (3) found that this factor can be preserved by suitable methods of ensiling and that cows fed such silage produced a milk rich in the factor. In this paper are reported additional data on the effectiveness of various methods of ensiling for preservation of the factor and also data on the growth-promoting quality of the milk from cows fed a number of these silages.

EXPERIMENTAL

Guinea pigs weighing approximately 300 gm. each were fed a basal diet of mineralized milk supplemented with riboflavin as reported in the previous publication (3). This milk was obtained from cows that had been fed for several months a winter ration which was low in the grass-juice factor.

Silages. The silages fed were preserved in the various ways listed in table 1. Three types of containers were used: quart milk bottles, 40 and 50 gallon barrels, and regular silos.

Samples of the fresh forages at the time of cutting were dried 24 hours at 40° C., ground and stored in the refrigerator. When the bottles, barrels, and silos were opened, samples of the silages were dried, ground and stored in the same way.

Guinea pigs, usually two or more animals per group, were fed 3 gm. per day of the dried materials as a supplement to the basal diet. Orange juice was added to the dried silages to increase the palatability. Weight gains of animals receiving these supplements are given in table 1.

Milks. The growth-promoting qualities of milk produced by cows fed some of the preceding silages were determined. Three groups of five cows each were included in the experiment. Groups I and II each contained two Holsteins, two Guernseys and one Brown Swiss; Group III consisted of two Holsteins and three Guernseys. The ration was 39 lbs. of silage, 8 lbs. of alfalfa hay, and 8 lbs. of grain mixture per cow per day. Oats-peas silage made in different ways was fed to group I for varying periods of time as

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follows: molasses-preserved silage, 4 months; untreated silage, 5 weeks; phosphoric-acid-treated silage, 7 weeks. Alfalfa silage prepared with 20 lbs. of phosphoric acid per ton was fed to Group II for 4 weeks, the same forage prepared with 14 lbs. of acid per ton was fed for 5 weeks, and that with 8 lbs. of acid per ton was fed for 10 weeks. Group III received molasses-alfalfa silage for 5 months.

Each day all the milk from one milking of the cows in a group was mixed together, and a quart sample was taken. This sample was fed to a group of 3 or 4 guinea pigs. A small amount of the milk plus 1 mg. iron, 0.1 mg. copper and 0.1 mg. manganese was fed first in the morning, and after this had been consumed, an excess of milk was given. The average growth curves are plotted in figure 1. The growth curve of a control animal fed the basal winter milk is also included in the figure.

DISCUSSION

The increases in weight of the guinea pigs (table 1) show that the grass-

TABLE 1

Assay of silages for grass juice factor

Forage ensiled	Preservative used in ensiling		Weight gain of guinea pigs in 7 weeks
	Kind	Amount per ton	
			gms.
None	....	....	−30
Alfalfa	Fresh (not ensiled)	....	123
Alfalfa	None	....	22
Alfalfa	Molasses	60 lbs.	47
Alfalfa	Salt	10 lbs.	44
Alfalfa	Phosphoric acid	15 lbs.	88
Alfalfa	Soured whey concentrate* equal to whey at	600 lbs.	141
Alfalfa	Whey powder	80 lbs.	61
Clover-timothy (1–1)	Fresh (not ensiled)	....	98
Clover-timothy (1–1)	None	....	16
Clover-timothy (1–1)	Molasses	60 lbs.	50
Clover-timothy (1–1)	Prosphoric acid	30 lbs.	93
Oats-peas (1–1)	Fresh (not ensiled)	....	76
Oats-peas (1–1)	None	....	36
Oats-peas (1–1)	A.I.V. acid mixture	34 litres 2N acid	74
Oats-peas (1–1)	Molasses	60 lbs.	89
Oats-peas (1–1)	Phosphoric acid	20 lbs.	108
Soybean	Fresh (not ensiled)	....	155
Soybean	None	....	114
Soybean	Molasses	100 lbs.	103
Sudan grass	Fresh (not ensiled)	....	93
Sudan grass	Molasses	40 lbs.	70

* Soured by *L. bulgaricus.*

juice factor of the forage was retained in varying degrees by different methods of ensiling. Acid-prepared silages were somewhat superior to molasses-

preserved silages in growth-promoting quality. With the exception of soybean silage, silages prepared without added preservative were rather low in the grass juice factor. Alfalfa preserved with soured whey concentrate gave especially good growth. Of the forages tried, soybean was the richest in the grass-juice factor.

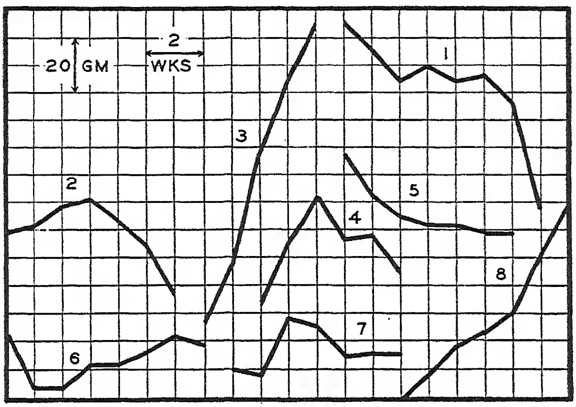

FIG. 1. Average growth curves of guinea pigs receiving various mineralized milks as follows: No. 1, control basal winter milk; No. 2, molasses alfalfa silage milk; No. 3, phosphoric acid alfalfa silage milk (20 lbs. acid per ton); No. 4, as No. 3 at 14 lbs. per ton; No. 5, as No. 3 at 8 lbs. per ton; No. 6, molasses oats-peas silage milk; No. 7, no preservative oats-peas silage milk; No. 8, phosphoric acid oats-peas silage milk (12 lbs. acid per ton).

From figure 1 it can be seen that good growth was obtained with milk produced from alfalfa silage that had been prepared with 20 lbs. of phosphoric acid per ton, but the use of smaller amounts of phosphoric acid resulted in poor quality milks. As reported elsewhere (4) better preservation of other constituents in the silage was obtained with the higher amount of phosphoric acid. With oats-peas 12 lbs. of acid per ton was sufficient to insure the presence of the factor in the milk but when this forage was ensiled with molasses, the milk produced from it was of low potency.

SUMMARY

The grass-juice factor of forages can be preserved in silage but the extent of preservation varies with different methods of ensiling. Silages prepared with phosphoric acid contained somewhat more of the factor than molasses-treated and untreated silages. Addition of soured-whey-concentrate to alfalfa gave excellent preservation.

The quantity of grass-juice factor in winter milk was increased by feeding silages rich in the factor, *e.g.*, silages preserved with adequate amounts of phosphoric acid.

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THE RELATIONSHIP OF pH TO SOME CURD CHARACTERISTICS OF MODIFIED MILKS

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In recent studies of the digestibility of milk several *in vitro* tests have been proposed in which stress has been laid upon the property of the curd particle size or the curd surface area. In the Chambers-Wolman test (1, 2, 3) the curd surface area is the index by which digestibility is gauged. In other methods reported by Hull (4) and Flora and Doan (5) although the measurement of protein degradation has been the basis of determining digestibility the importance of the size of the curd particles has been evident.

Among the factors which may influence the type of curd formation the pH at which coagulation occurs and the range of pH throughout digestion are of significance. This has been noted by several investigators (3, 4, 6). In the *in vitro* tests suggested, however, there have been considerable differences in technique with respect to the pH levels employed and possibly this has been a matter of greater controversy than any other single factor.

Available published reports concerning the conditions of acidity in the human stomach offer little to clarify the situation. The methods employed and the results obtained by several workers (6 to 15 inclusive) have differed so widely that the data appear too variable to warrant the formation of any definite conclusions.

Thus if the effect of pH on the coagula of different types of milk was proportionately the same it would make little difference what pH level was selected for *in vitro* tests. Preliminary work by the author indicated that some modified milks might react differently than others to pH changes. This investigation was undertaken to ascertain the behavior of various commercially modified milks at different pH levels.

EXPERIMENTAL

Samples: All samples used in the study were commercially prepared and included the following types of milk:

Untreated milk, both raw and pasteurized.
Homogenized milk (high pressure, piston type homogenizer).
Enzyme-treated milk (pancreatic enzyme extract).
Base exchange milk.
Evaporated milk, diluted 1:1 with water.

Curd surface area measurements: The Chambers-Wolman test (1, 2) with the modified technique as described by Anderson (3) was used for measurements of curd surface area. The samples were coagulated in thin-

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walled latex sacs, using sufficient coagulant to adjust each to the desired pH level. The coagulant consisted of a mixture of equal parts of 0.6 per cent pepsin solution (U.S.P. 1: 3000) and normal hydrochloric acid. After a digestion period of thirty minutes with constant agitation the samples were emptied into individual containers and hardened with formaldehyde. After sieving and weighing the various size fractions of curd particles the relative surface area per gram of curd (S/gm.) was determined by calculation. The results reported are the averages of duplicate tests.

Measurements of total curd formation: In the Chambers-Wolman test the measurement of the total amount of curd formed is a necessary step in the calculation of the curd surface area. The same data were used in this investigation as a method of studying the relative bulkiness and completeness of curd formation at different pH levels. As a further guide to the completeness of coagulation the appearance of the samples at the end of the "digestion" period was observed. Complete coagulation was considered to have occurred only when there was a definite and complete separation of the sample into coagulum and whey.

pH values: A Beckman pH meter with remote electrodes was used and all readings were made at a temperature of 25° C. Each sample was tested at the following pH levels: 6.0, 5.5, 5.0, 4.5 and 4.0.

Total solids: Total solids were determined by means of a lactometer and butterfat tests.

All tests on any given sample of milk were performed on the same day.

RESULTS

Figure 1 shows the average amounts of curd recovered from the different types of milk in the Chambers-Wolman test throughout a pH range from 6.0 to 4.0.

The untreated, homogenized and enzyme-treated milks yielded somewhat similar results with the smallest amount of curd being recovered in each case at pH 5.0. Of these three types of milk the homogenized varied the least and tended to form a somewhat bulkier curd throughout the entire range. The amount of curd formed by the untreated milk was greatest at pH 6.0, dropped to its lowest point at pH 5.0 and then increased slightly as the pH was lowered further. The enzyme-treated milk showed the greatest variation and formed considerably smaller amounts of curd than the other two at pH 5.5 and pH 5.0. As judged by the amounts of curd recovered and the appearance of the samples these milks all coagulated completely throughout the pH range studied.

In base exchange and evaporated milk the effect of incomplete or partial coagulation was noticeably demonstrated. It is an established fact that these milks do not form any appreciable curd under the conditions of the curd tension test which is carried out at about pH 6.0. Likewise, when the

Chambers-Wolman test was performed at pH 6.0 the base exchange milk averaged 7.14 grams of curd while the evaporated milk did not form any curd. As the pH was lowered both types coagulated completely. In the case of base exchange milk this point was reached at about pH 5.5 and with evaporated milk at about pH 5.0.

Table 1 shows a comparison between the total solids content and the average amounts of curd recovered. This was done as a check against the effect of possible variations of the total solids of the different milks upon the amounts of curd formed in the Chambers-Wolman test. It is evident from the data that there was little relationship if any between the solids content of the milks and the amounts of curd recovered.

TABLE 1

The average total solids and the amounts of curd recovered in the Chambers-Wolman test at different pH levels

Type of milk	No. of samples	Total solids	Amount of curd recovered				
			pH 6.0	pH 5.5	pH. 5.0	pH. 4.5	pH 4.0
		%	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Untreated	11	13.47	40.98	34.35	27.79	31.50	35.33
Homogenized	10	12.98	42.37	36.52	36.07	37.95	40.17
Enzyme-treated	11	13.03	39.21	26.25	23.17	33.62	38.50
Base exchange	10	12.57	7.14	30.13	30.47	32.33	39.08
Evaporated (1:1)	10	13.78	0.00	27.64	52.54	54.17	52.09

In figure 2 are shown the average values for curd surface area throughout a pH range from 6.0 to 4.0. Also in this respect the untreated, homogenized and enzyme-treated milks followed the same general pattern with the values being low at pH 6.0 and increasing as the pH decreased. At pH 6.0 and 5.5 all three types of milk gave approximately the same low values. It was only at pH 5.0 or lower that there were appreciable differences.

The results with base exchange milk differed from the three just mentioned in that the curd surface area was comparatively high at pH 6.0 and dropped to its lowest point at pH 5.5. Undoubtedly this high surface area was due to partial coagulation of the milk. At pH 5.0 the curd surface area was approximately equal to that of the untreated milk and, while it increased somewhat as the pH decreased, base exchange milk still had the lowest surface area of all samples at pH levels of 4.5 and 4.0.

With respect to curd surface area the evaporated milk was superior to all types tested. No curd was formed at all at pH 6.0 while at pH 5.0 the values were generally very high. As with base exchange milk the high surface area at the upper pH levels coincided with partial coagulation. The lowest curd surface area occurred at pH 5.0 from where it increased slightly at pH 4.5 and showed a much greater increase at pH 4.0. Five different brands of canned evaporated milk were included in the study. There ap-

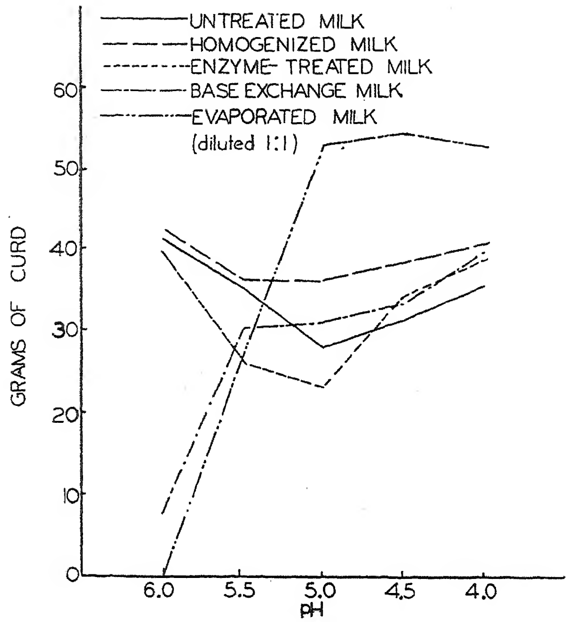

FIG. 1. The total amounts of curd recovered in the Chambers-Wolman tests throughout a pH range from 6.0 to 4.0. (Averages of all samples of each type of milk.)

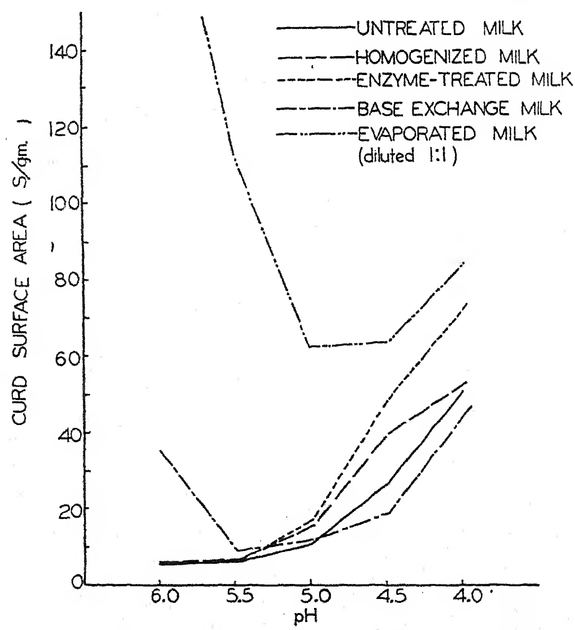

FIG. 2. The average curd surface area of modified milks throughout a pH range from 6.0 to 4.0.

peared to be some similarity of results within brands. However, the numbers of samples of each brand tested were too small to permit the formation of definite conclusions.

DISCUSSION

Considering the data with respect to the total amounts of curd formed in the Chambers-Wolman test it becomes apparent that there are two principal factors which will affect the results: 1) the completeness of coagulation and 2) the hydration of the curds or their affinity for water. Since there were no adequate means available for measuring either of these in a quantitative manner no attempt to do so was made in this investigation.

The completeness of coagulation was judged almost entirely by the appearance of the samples after "digestion" and was interpreted as being "no coagulation," "partial coagulation" or "complete coagulation" depending upon whether there was no curd formation, some curd formation or a complete separation of curds and serum. Under the conditions of the tests incomplete coagulation was observed only with base exchange and evaporated milks within the pH range studied. While the untreated, homogenized and enzyme-treated milks all coagulated completely at pH 6.0 and lower, it seems logical to expect that if even higher pH levels had been employed a zone would have been found in which they too would have exhibited varying degrees of partial coagulation. This statement is based upon the assumption that if coagulant were added to the milk in increasing increments it would be expected that coagulation would occur gradually.

The values reported as the total amounts of curd recovered in the Chambers-Wolman test are "wet" weights and therefore any variations in the hydration of the curd formations would have a corresponding effect upon results. While no specific method of measuring the degree of hydration was employed there seems to be no other logical explanation that could be offered for the variations in the amounts of curd formed at different pH levels in those samples wherein coagulation was complete. Certainly the total solids content (Table 1) was of little importance and it is not likely that at the pH levels employed there was any actual peptic digestion of consequence. With respect to the effect of pH upon the total curd formation, the general reaction seems to be determined by the inherent physical and chemical properties peculiar to each type of milk as a result of the method of modification employed.

When considering tests run throughout a range of pH levels there seemed to be a general relationship between the amount of curd formed in the Chambers-Wolman test and the curd surface area. This was particularly true at the upper pH levels where coagulation of the samples was more likely to be incomplete. Attention has been called to the effect of partial coagulation upon the values for curd surface area in the case of the base exchange and evaporated milks. It is interesting to observe that after these milks had

coagulated completely their values for surface area increased with a decrease in pH in much the same manner as the untreated, homogenized and enzyme-treated milks. Therefore, in the relationship of pH to curd characteristics one fact seems to hold true for all milks, *i.e.*, the curd surface area is lowest at the highest pH level at which complete coagulation will first occur. In the case of the untreated, homogenized and enzyme-treated milks this point is apparently reached somewhere above pH 6.0 and with base exchange and evaporated milk it is at about pH 5.5 and 5.0 respectively. Also, at any pH below that required to bring about complete coagulation, the curd surface area increases as the pH is lowered.

It has already been mentioned that there has been considerable variation in the *in vitro* techniques used by several investigators. This probably has been due to a lack of definite and conclusive knowledge of the coagulating conditions within the stomachs of human beings. Inasmuch as wide variations in the coagulating characteristics of milks occur within the pH limits thus far reported there does not seem to be any single pH level that is satisfactory for comparative tests on all milks.

CONCLUSIONS

In the Chambers-Wolman test the curd surface area of any milk appears to be lowest at the highest pH level at which complete coagulation will first occur. At any pH below that required for complete coagulation the curd surface area increases as the pH is lowered.

The effect of pH upon the bulkiness or completeness of curd formation is variable in milks modified by different processes. The method of modification seems to be the most important factor in determining this relationship.

With our present knowledge there does not seem to be any single pH level suitable for comparative *in vitro* tests on all milks.

ACKNOWLEDGMENT

The author is indebted to Professor E. O. Anderson of the Department of Dairy Industry, University of Connecticut, for his many helpful suggestions during the course of this investigation.

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OBSERVATIONS ON DELAYED SALTING OF BRICK CHEESE[1]

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A few Brick cheese manufacturers have attempted to hasten the ripening of Brick cheese by delaying for several days the normal salting operation which usually occurs in the morning of the day following manufacture. Brick cheese must be salted after it is shaped into its characteristic form. Salting is accomplished by exposing the surfaces of the loaves to sodium chloride brine or to dry salt.

Examination of the literature on the salting of cheese shows that the flavor, body, texture and color of cheese, its rate of ripening and its composition can be affected by the method of salting, the amount of salt used, and by the time of salting.

The flavor of cheese can be affected unfavorably by unusual salting methods. If the cheese lacks salt, undesirable flavors may be produced (1, 4, 14). Bitterness in cheese has been attributed to a protein decomposition product, the presence of which can be traced directly to the salt content (20). It has been suggested (13) that the inhibiting effect of salt on the growth of lactic streptococci might explain the relation between salt concentration in cheese and cheese quality. The flavor developed during curing can be decreased by over-salting (1, 9, 17).

The body of cheese is sensitive to variations in salt content. Generally, over-salting tends to produce a hard, harsh body (1, 7, 17) while under-salting gives a pasty, weak body (1, 17). There is a range of concentration through which the salt content can be varied without noticeably affecting the body of the cheese (9); this range probably will vary with other factors affecting body such as type of cheese, moisture content and acidity. Studies of the peptizing effect of salt on rennet casein under different conditions of salt concentration and acidity indicate that the smoothness of Cheddar cheese should be favorably affected by the action of the salt on the paracasein in the pH zone between 5.5 and 6.0 (18). On the other hand, the fact that cheese protein is 100 per cent soluble in 3 to 10 per cent sodium chloride solutions within 7 days after making and remains soluble throughout the life of the cheese seems to justify the conclusion that the influence of salt on cheese quality cannot be caused by variation in the solubility of the protein in brine (12).

The texture of cheese can be made open by light salting and close by heavy salting (1, 4, 17). A white discoloration may be induced in some soft,

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ripened types of cheese by over-salting (14), and a similar fault has also been observed in Brick cheese (1).

Salt affects organisms inside and on the outside of cheese. Bacterial growth inside the cheese is delayed by early salting (3) and by variations in the amount of salt incorporated (4, 14). The presence of salt on the outside of types of cheese not unlike Brick seems to encourage the development of surface flora (7, 8).

The method of salting may influence the results obtained. Dry salting apparently reduces the weight of the cheese more than brine salting (2) and is not apt to be as uniform as brine salting (1). Salting cheese on the outside by either of these methods after the cheese is formed produces results not observed when the curd is salted before it is shaped (9).

The effects of salting on cheese characteristics may be associated with the rate of salt penetration. It has been observed that in Edam cheese 15 cm. in diameter the salt penetrated 5.5 cm. in 10 days (22); in Limburger, 8 to 10 days are required for practically uniform distribution (5, 15). Brine salted, whole milk Trappist cheese weighing 1.2 to 1.3 kg. showed uniform salt distribution in 60 days (16); and salt applied to the surface of Brick cheese requires approximately 8 weeks before it is uniformly distributed (1). Salt diffuses slowly in Cheddar cheese and penetrates most easily along the "grain"; within 12 hours after hooping the salt is essentially uniform (11), although the distribution of salt in cheese from the same vat may be surprisingly lacking in uniformity (10).

Some observations have been made on the influence of the time of salting certain types of cheese. In one study (3), one portion of curd was salted as soon as it was drained while identical curd was pressed 2 days and then salted in a brine bath. The early salting retarded bacterial development; delayed fermentation of lactose and acid development; and when there was incorporated more than 4 per cent of salt in the moisture of the cheese the cheese became hard, brittle and crumbly. The cheese salted after 2 days could absorb 8 per cent salt in the water of the cheese without injury. Salting curd 3 hours after draining gave results similar to those obtained by salting after 2 days. Other workers (8) reported that salting of Camembert cheese 1 day after making increases the dry matter; delays acid formation; increases the salt content; and hastens development of surface flora as compared to salting 2 days after making. The late salting causes slow salt penetration and, because the curd retains the whey, there is induced bitter flavor and whey sourness. When Brick cheese (1) is salted at 4 hours after dipping instead of the usual 20 hours, almost all of the characteristics of the cheese are affected. The acidity development is practically stopped, more moisture is retained; salt penetration is more rapid; and, although the flavor is not materially altered, the body of the early salted cheese tends to be curdy and hard and the texture is closer.

It is clear that salt influences the characteristics of cheese. The growth of organisms is affected by the salting treatments; the chemical substances produced in the course of the ripening process are determined in part by the salt; and the physical properties of the protein are influenced by the combined action of acidity and salt content. The smoothness of body observed after delayed salting treatments and the harshnes of body induced by early salting treatments are particularly significant in the making and curing of Brick cheese because this is a type of cheese which is most popular when the body has smoothness and good slicing properties. A few preliminary trials in this laboratory indicated that delayed salting induced differences which should be studied. The results of this study are reported here.

EXPERIMENTAL PROCEDURES

The manufacturing process used in these experiments was essentially that recommended by Spicer and Price (19). The experiments were made with raw and with pasteurized milk. Milk cultures of *S. lactis* were used for starters and a cooking temperature of 104° F. was therefore adopted.

The loaves of cheese from each lot of milk used in these experiments were divided into groups before salting. The cheese made from raw milk was divided into two groups one of which was salted in the usual manner on the day after making while the experimental group was salted on the 5th day after making. The cheese made from pasteurized milk was divided into three groups; the first or control group was salted the day after making; the second was salted on the 5th day; and the third group was salted on the 9th day after making. Each group was salted by holding in 23 per cent sodium chloride brine for 48 hours. All groups were kept at approximately 60° F. during salting, during the usual washing and until paraffining. All groups were paraffined on the 14th day after making and were then held at approximately 50° F. until the final grading.

Analyses for moisture (21) and acidity (pH) were made at 21 hours, just before salting, at paraffining and again when the cheese was 10 weeks old. Acidity measurements were made with a Leeds Northrup portable potentiometer using the quinhydrone electrode and saturated calomel half cell. Salt determinations (21) were made after salting and at paraffining and again when the cheese was 10 weeks old. These analyses were made by using the whole of a cross section slice of a loaf of cheese after discarding about ¼ inch of the rind layer. During the 5- and 9-day intervals before salting, the loaves of cheese were held in the 60° F. curing room and were moistened daily with water and rubbed to prevent mold growth.

All lots of cheese were graded at 14 days and again at 10 weeks of age.

RESULTS

The quality of the cheese is shown by the average grades listed in tables 1 and 2.

In table 1 are shown the grades of the 5 lots of cheese made from raw milk that were subjected to the different salting treatments. At two weeks of age the average quality of the cheese salted on the first day after making was practically identical except perhaps in flavor to that of the cheese salted five days after making. After 10 weeks of aging there was evident a slight margin of difference in favor of salting on the first day after making. The quality of the milk used in these experiments was not very good. All lots of cheese were criticized for off, sharp or unclean flavors after 2 weeks and for very unclean and strong flavors after 10 weeks of curing, regardless of the salting treatments.

TABLE 1

Effect of delayed salting on the quality of five lots of raw-milk cheese

Characteristic	Time of salting	
	1st day	5th day
	Average grades* at 14 days of age	
Flavor	3.5	3.7
Body	2.4	2.5
Texture	3.7	3.6
	Average grades* at 10 weeks of age	
Flavor	3.4	3.8
Body	3.2	3.4
Texture	3.2	3.4

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectionable; 5 = Very Objectionable.

TABLE 2

Effect of delayed salting on the quality of three lots of pasteurized-milk cheese

Characteristic	Time of salting		
	1st day	5th day	9th day
	Average grades* at 14 days of age		
Flavor	2.7	2.7	2.3
Body	2.0	2.3	1.3
Texture	2.5	2.7	1.8
	Average grades* at 10 weeks of age		
Flavor	3.3	3.0	3.7
Body	2.7	2.3	2.3
Texture	2.7	2.3	2.3

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectionable; 5 = Very Objectionable.

Early gas, evidently caused by organisms of the Escherichia-Aerobacter group, was present in every lot of raw-milk cheese regardless of the salting treatment. No definite relation could be observed between the time of salting and the degree of openness in the texture of the cheese. Despite the fact that lack of salt during the first 2 weeks of curing encourages the development of the splitting defect (4) in Brick cheese, this fault was not observed in the raw-milk cheese. The judges found a softness of body in the raw-milk cheese

salted 5 days after making which was not apparent in the cheese salted the day after making. This softness was of such a character that it was regarded as a defect.

The data of table 2 show the grades given the groups of cheese made from pasteurized milk. The grading of the cheese when it was 14 days old showed little difference in quality between those groups salted on the first and 5th day after making. The cheese salted on the 9th day was definitely better than that in the other groups. This improvement was found chiefly in a very desirable smooth, long body and a closer texture in the cheese. The delay in the salting operation for 9 days seemed to cause a more rapid disappearance of the normal curdy characteristics of the cheese. There was a slight improvement in flavor which was evident as a sweet or Swiss-like aroma in some of the lots salted 9 days after making.

After 10 weeks of curing the differences in quality between the groups observed when the cheese was younger had practically disappeared. The quality of all groups of cheese was regarded less favorably by the judges. These trends can probably be attributed to the quality of the original milk from which the cheese was made. The defects observed in the flavor were found in all three groups of cheese but the cheese salted 9 days after making had deteriorated most markedly. Regardless of the salting treatment all groups of cheese showed some mealiness of body. The texture of the cheese salted at 5 and 9 days after making was inferior to that of the control lot. Especially significant was the fact that the cheese salted on the 9th day showed some splitting.

The differences in quality recorded in tables 1 and 2 as the result of delayed salting might be caused by either biological or chemical changes or, more probably, both. There can be little doubt that delayed salting permitted the early and rapid growth of organisms which are ordinarily suppressed by the presence of salt. The marked differences in the body of the cheese, especially in the early stages of curing, reflect the effect of salt on the physical and chemical changes in the protein. It is well known in the industry that salt causes a firmness in cheese that cannot be attributed entirely to a lowered percentage of moisture. In addition to this effect, differences in the experimental cheese may be caused indirectly by the probable influence of salt upon normal acidity changes which must precede the breakdown of cheese curd.

The amount of salt in the cheese. The total amount of salt present in the cheese 10 weeks after curing was only slightly affected by the delay in salting as shown in table 3. The slightly higher concentration in the control lots can be explained by the use of 10 per cent sodium chloride brine to moisten the cheese during the interval of "smearing" before paraffining. Those lots given the delayed salting treatment received brine rubbing only after they had been salted; before salting only fresh water was used on them

for smearing purposes. It seems apparent that delaying the salting treatment influences the final salt content of the cheese so little that differences in the cheese must be primarily associated with the time interval between making and salting.

TABLE 3

Effect of delayed salting on the salt content of Brick cheese

Time of analysis	Time of salting		
	1st day %	5th day %	9th day %
	Raw-milk cheese*		
After salting	1.43	1.06	....
At 14 days	1.90	1.76	....
At 10 weeks	1.90	1.82	....
	Pasteurized-milk cheese**		
After salting	1.12	1.08	0.84
At 14 days	1.66	1.67	1.56
At 10 weeks	1.73	1.67	1.67

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

Cheese acidity. The effect of delayed salting on the acidity of the cheese is shown in table 4. These results if considered alone would lend support to the belief that delayed salting would hasten the ripening process. The curing of cheese is accompanied by an increase in pH value; such results are slightly apparent in the trend of data shown for the pasteurized-milk cheese and a little more apparent in the data on acidity changes in the raw-milk cheese. The differences are so small however that they can be disregarded, especially in view of the meager supporting evidence obtained in examining the quality of the cheese.

The pH values shown for the raw-milk cheese are higher at the 10-weeks' interval than those shown for the pasteurized-milk cheese. This result is to be expected in view of the decreased biological activity in the curing process following the heat treatment of the milk.

Losses of moisture during curing. The effects of the delayed salting on moisture losses during the curing process are shown in table 5. There is normally a downward trend in the moisture content of Brick cheese during the first 14 days of curing. This is caused by evaporation losses and by the incorporation of salt. The salt has a double effect in that it tends to decrease the water-holding capacity of the curd and because, as it is absorbed, it increases the dry matter. The combination of these effects as shown in the data of table 5 indicates that the delayed salting of the curd increased the losses of moisture between making and paraffining at 14 days of age. After paraffining the losses were practically identical regardless of the previous

TABLE 4

Effect of delayed salting on the cheese acidity

Time of observation	Time of salting		
	1st day pH	5th day pH	9th day pH
	Raw-milk cheese*		
Before salting	5.08	5.10	....
After salting	5.03	5.17	....
At 14 days	5.25	5.26	....
At 10 weeks	5.39	5.43	....
	Pasteurized-milk cheese**		
Before salting	5.08	5.06	5.14
After salting	5.05	5.08	5.13
At 14 days	5.13	5.17	5.19
At 10 weeks	5.20	5.26	5.27

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

salting treatment and regardless of whether the cheese was made from pasteurized or raw milk. It seems probable that the softening of the body,

TABLE 5

Effect of delayed salting on losses of moisture

Losses during the interval:—	Time of salting		
	1st day %	5th day %	9th day %
	Raw-milk cheese*		
Before salting	0.0	1.2	......
During salting	2.6	2.2	......
Salting to 14 days	0.6	0.7	......
14 days to 10 weeks	0.6	0.6	......
Total moisture losses	3.8	4.7	
	Pasteurized-milk cheese**		
Before salting	0.0	0.8	1.1
During salting	1.6	2.3	1.7
Salting to 14 days	1.4	0.5	0.7
14 days to 10 weeks	0.9	1.0	0.9
Total moisture losses	3.9	4.6	4.4

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

observed in the lots of cheese salted on the 5th and 9th days after making must therefore be attributed to protein changes rather than to the retention of more moisture.

CONCLUSIONS

Delayed salting as practiced in these experiments seems to have no real benefits to commend it to the practical operator. There is an apparent improvement in the body of the cheese at the end of the 14-day period during which the cheese is retained in the factory. By the time the cheese has been cured, however, this benefit has disappeared and the general quality of the resulting cheese is not as good as that of the cheese salted in the normal manner. The addition of salt soon after making probably establishes a desirable trend in flavor production and body changes in Brick cheese curd that does not happen when salting is delayed.

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THE RELIABILITY OF THE ROOM TEMPERATURE HOLDING TEST AS AN INDEX TO THE KEEPING QUALITY OF BUTTER

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The "room temperature holding test" is understood to include all butter keeping quality tests carried on by incubating small samples of butter for periods of from 6 to 10 days at temperatures of from 67° to 70° F. The actual practices in the various plants differ as to time and temperature but the general idea of predicting the keeping quality of butter in commercial channels on the basis of the flavor and odor developed at relatively high temperatures is common to all.

The need for such a test has been emphasized by a study made by Sprague, Foelsch and Small (1) of the butter offered on the large metropolitan retail markets. The survey was made of selected brands sold in one-pound cartons in New York and Chicago. In their conclusions the investigators stated that the instances in which deterioration had lowered the score more than one full point from the original score were few but still numerous enough to indicate that keeping quality is a serious problem. They state further that "For the purpose of identification of butter which lacks keeping quality and for the prevention of its use in cartons carrying certificates of quality, a wider use of incubation tests for keeping quality is desirable."

The relation of the time elapsed between grading and purchase and the loss in score showed that the time involved in the actual handling and sale of fresh butter supplies ranged up to 25 days, with an average of about 15 days. Since the butter was exposed to many temperature changes during transportation and finally in the retailers hands, the problem of stable butter quality was shown to be a very important factor in marketing.

PREVIOUS WORK

The prediction of the keeping quality of butter in ordinary commercial channels has been given increased attention in recent years probably due to the tendency toward lightly salted butter of higher flavor quality.

Hunziker (2) described two keeping quality or incubation tests involving holding small portions of butter at room temperature or higher. He stated that the tests are effective in revealing relative resistance of butter to flavor deterioration due to bacterial causes. He emphasized the use of the test in the prevention of surface taint by detecting fermentation in the small portions held in the test.

The value of the keeping quality test in the detection of faulty methods

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of production was indicated by Hammer (3). In a discussion of the development of methods of making keeping quality tests he emphasized the fact that the test probably can detect only those defects which are due to organisms rather than those due to chemical action. He states that "Temperature has a definite effect on the growth of organisms in butter, and a close correlation between the deterioration at various temperatures cannot be expected."

The use of a test involving parchment wrapped print butter was described by Parsons (4). The butter was held for fourteen days at 60° F. in a room of controlled humidity of 90 to 100 per cent. He found the test useful in the detection of butter of uncertain handling quality. He indicated that a test involving eight days at 70° F. gave equivalent results but that it required closer temperature control to prevent "oiling off" of the butter in certain seasons.

Sorensen (5) reported on an extensive survey of the keeping quality of both salted and unsalted butter in commercial channels by the use of the holding test. One-fourth pound parchment wrapped samples were held in a thermostatically controlled cabinet at 68° to 70° F. for seven days. The samples were examined for flavor and odor defects at the end of this period and reported as satisfactory, fair or unsatisfactory. A total of 22,060 churnings were examined representing more than 100 plants in eight midwestern states. The author states "a surprisingly close correlation between keeping quality tests and subsequent difficulty with the churnings tested was noted." The putrid-cheesy type of flavor defect was the most frequently encountered defect in the salted butter which showed unfavorable keeping quality. The value of the keeping quality test in locating contaminated water supplies or unsanitary plant conditions was pointed out.

Previous work at this station (6) has shown the relation between the numbers of bacteria in butter and the keeping quality at various temperatures. Similar high points in numbers of bacteria were developed in the incubation test for 7 days at 70° F., after 3 to 4 weeks at 40° F. and after 8 weeks at 32 to 36° F. The types of bacteria growing at these different temperatures varied and as might be expected the type of flavor varied with the holding temperature. It was found that lipolytic and proteolytic bacteria grew best at 40° F. as indicated by the fact that the counts reached higher levels at this temperature than at either the higher or lower temperatures. At 70° F. the lactic acid forming organisms usually predominated and their activity, no doubt, inhibited the growth of more objectionable types. The sour flavor developed at this temperature frequently was sufficient to mask other off flavors which were present and which would develop at lower temperatures.

THE PROBLEM

The holding test has been applied by many of the large butter manufacturers in recent years with good success. There has been a feeling on the

part of the operators, however, that the results of the keeping quality or incubation test has failed in certain cases to sort out properly all of the butter which was of uncertain quality. In some cases butter which failed to show definite deterioration in the incubation test would break down before it could reach the ultimate consumer while in other cases the deterioration developed in the incubation test was of a type which did not occur under the temperature conditions commonly found in butter warehouses and retailers' holding rooms. It was to obtain some information on possible causes of this lack of agreement that the following work was done.

PROCEDURE

The butter in this study was obtained from the educational scoring contests held at the station over a period of three years and included 78 lots representing 25 different creameries in South Dakota. The butter represented regular commercial churnings in some cases while in others it was made especially for the contests. All of the butter was salted with the salt content ranging from 0.5 per cent up to 3.0 per cent and averaging 1.8 per cent.

Samples were obtained with sterile spatulas and placed in 5-ounce glass jars with screw tops protected by parchment paper liners. Two samples were obtained from each tub, one to be held at 70° F. in a thermostatically controlled box, and one at 40° F. in the laboratory refrigerator. These lots were scored and examined for bacteria, yeast and mold when fresh and at intervals during the holding period. The scoring and microbiological analysis were done after 7 days at 70° F. and after 28 days at 40° F. All samples were tempered overnight to approximately 40° F. before scoring regardless of the temperature of holding to make the results of scoring more comparable.

The fresh butter scores were made by the official judges of the contests and by members of the department while the held butter was scored and criticized by members of the dairy department. The yeast and mold and bacteriological studies were made according to the methods suggested by the American Dairy Science Association Committee on Microbiological Analysis of Dairy Products (7).

RESULTS

In order to show the application of the Holding Test to the grading out or sorting out churnings of questionable keeping quality, the 78 lots were divided according to loss in score. In table 1 two methods of classifying the butter are presented. The average scores of the butter when fresh and the loss in score after holding are shown to permit a comparison of the keeping qualities of the two lots.

The results in table 1 under method A show the division of the butters into those showing less than one point loss in the holding test and those

TABLE 1

The comparative keeping quality of butter classified by the holding test

Method	Holding test Loss in score	Number of lots	Fresh score	Loss in score Holding test 7 days—70° F.	Loss in score 1 mo.—40° F.
A	less than 1 point	38	91.68	.10	.28
	1 point or more	40	92.36	2.12	1.44
B	1 point or less	55	91.92	.42	.58
	more than 1 point	23	92.32	2.80	1.91

showing one point or more loss. This method divided the 78 lots almost evenly. A slightly lower average fresh score was obtained in the class which showed less than one point loss. This might be expected because the deterioration in flavor score can be noted more easily when higher scoring butter is involved. The effectiveness of the holding test is indicated by the close relation between the holding test loss in score and the loss in score at 40° F.

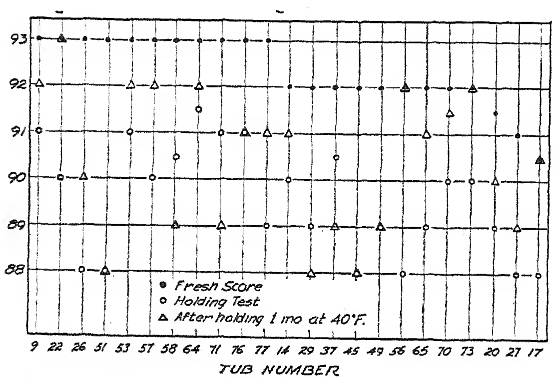

FIG. 1. Relation of "holding test" score to fresh score and to score after holding 1 month at 40° F.

(Lots losing more than 1 point in holding test)

Under method B in table 1 the butters were divided into two groups with only those showing more than one point loss being placed in the low keeping quality group. This division resulted in a smaller number of lots going into the low keeping quality group but the relation between groups was not greatly altered. The twenty-three lots which lost more than one point in the holding test were arranged in Fig. 1 according to the fresh butter scores to show the relationship between the holding test and the score after one month at 40° F. The results with individual lots are shown here to permit a study of the agreement between the test and the score of different lots after holding. In most cases the loss in score in the holding test and a loss during one month at 40° F. compare fairly well. In certain cases such as tubs num-

ber 22, 56, 73, and 17 losses in the holding test were not borne out in the butter held at the lower temperature. In three of these lots, cheesy or rancid flavors developed at room temperature but were not detected at the lower temperature within one month. A longer period at 40° F. might have brought out the flavor deterioration indicated in the holding test. As previously stated, however, it is probably more important to the butter manufacturer to know that the holding test finds the butters of uncertain keeping quality rather than to show absolute agreement with the record of each individual lot. It is apparent from these results that either method of classification results in a general segregation of those butters of low keeping quality from those of more satisfactory keeping quality.

There were exceptions to the general rule as indicated by the fact that seven of the fifty-five which lost one point or less in the holding test showed more than one point loss when held one month at 40° F. Six of these were given a score of 93 when fresh. Also eleven of the butters which lost more than one point in the holding test failed to show more than a point loss in the month storage at 40° F.

The value of the test in selecting butter of poor keeping quality is also indicated by a survey of individual lots in table 2 which showed that every lot which fell below 90 in score at the end of one month at 40° F. was classified in table 1 in the poor-keeping-quality group by the holding test. The greatest discrepancy between results appeared in those cases in which the holding test indicated poorer keeping quality than was found after holding at the lower temperature. Such lots as No. 17 and 56 which became cheesy at room temperature but held their original score at 40° F. were such cases.

The reason for such lack of agreement probably lies in the type of changes taking place in the butter at the different temperatures. The different types of flavors which developed under different holding conditions are shown in table 3. It may be noted that such flavors as feed, old cream and acidy were much more prevalent in the fresh butter than after holding. Flavors such as stale, cheesy or rancid were most marked after the room temperatures holding test, while the flavors developed after one month at 40° F. were storage, coarse, stale, oily and rancid. These results indicate that the room temperature holding resulted in more bacterial deterioration than the lower temperature holding which permitted chemical action but limited the bacterial action. The difference in the flavors produced appears to indicate this conclusion.

The study of the numbers of lipolytic or fat splitting bacteria and proteolytic or casein digesting bacteria supported the viewpoint expressed above. These types of organisms were absent in most of the plates made from fresh butter but after 7 days at room temperatures, large numbers of proteolytic bacteria were sometimes found. The presence of these types was found to be associated with the development of cheesy flavor in most cases. At the

TABLE 2

The "Holding Test" and loss in score after one month at 40° F.

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
1	92	old cream	92	sl storage	92	sl storage
2	92	old cream	92	coarse	91	stale
3	92	sl oily	91	sl stale	91	stale, briny
4	93		92	flat	92	
5	93		92	chem	92.5	
6	92	sl feed	92	briny	92	briny
7	93	sl bitter	92	flat, briny	91	stale
8	91.5	old cream	92		91.5	sl stale
9	93		91	tallowy	92	
10	92.5	sl bitter	92	coarse	92	coarse
11	91	bitter	91	tallowy	91	sl stale
12	93		93		92	sl stale
13	93		92	sl stale	92	sl stale
14	91.5	old cream	90	sl cheesy	91	stale
15	92	old cream	92	sl stale	91.5	sl storage
16	91	briny, old cream	91	coarse	91	coarse
17	90.5	briny, old cream	88	sl cheesy	90.5	old cream
18	93		93		93	
19	93		92	sl acidy	93	
20	91.5	stale cream	89	unclean	90	stale
21	89	neutralizer	89	storage	89	storage
22	93	acidy	90	sl rancid	93	
23	93		93		93	
24	90	neut. weedy	89	stale	90	briny, weedy
25	91	neut. coarse	91	coarse	91	briny, grassy
26	93		87	rancid	90	flat, sl oily
27	91	cooked, sl uncl	88	unclean, acidy	89	unclean
28	93		93		91	storage
29	92	flat, sl old cream	89	moldy	88	sl rancid
30	93		92.5	flat	93	flat
31	93		92	sl storage	92	
32	91.5	old cream	92	coarse	91.5	storage
33	91	sl unclean	90	stale, sl rancid	90	storage
34	92	sl coarse, burnt	92.5		91.5	sl malty
35	91.5	acidy	91.5	acidy	91	briny, acidy
36	92	briny, acidy	91.5	briny	92	
37	92	sl acidy	90.5	acidy, stale	89	sl moldy
38	91	acidy	92	coarse	91	coarse
39	92	sl flat	93		92	
40	93		93		91	stale
41	92	briny, acidy	92.5		91	coarse
42	90	briny, metallic	91	briny	91.5	briny
43	92	acidy	91.5	storage	92	
44	92	acidy	92	acidy	91	sl flat
45	92	cooked	88	rancid	88	stale, fishy
46	91	burnt	91.5	stale	91	coarse
47	91	sl unclean	91	sl unclean	91	sl stale
48	92	sl acidy	92		91.5	briny
49	92	acidy	89	oily, acidy	89	stale
50	92	sl utensil	91	flat	93	
51	93		88	cheesy, oily	88	fruity, rancid
52	92.5	sl barny	92.5		92	coarse
53	93		91	sl fruity	92	sl stale
54	92	sl feed	91	sl unclean	92	

TABLE 2—(*Continued*)

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
55	93		92	flat, sl tallow	93	flat
56	92	utensil	88	cheesy, rancid	92	
57	93		90	stale	92	
58	93		90.5	bitter, metallic	89	rancid
59	90.5	stale, malty	92	flat	92	
60	90	met., burnt, neut.	91	sl tallow	91	old cream
61	93		92	sl storage	92	sl storage
62	93		93		93	sl coarse
63	91.5	sl musty	91	sl stale	90	oily, stale
64	93		91.5	sl stale	92	sl storage
65	92	wintery	89	sl cheesy	91	stale
66	93		92		91.5	coarse
67	90	burnt, metallic	92	coarse	91	briny, old cream
68	93	heated	92		90	oily, neut.
69	92	briny	91	sl stale	91	storage
70	92	sl coarse, feed	90	sl cheesy	91.5	coarse
71	93	sl heated	91	sl stale	89	woody
72	91	burnt, malty	92	sl bitter	91	burnt, old cream
73	92	coarse, briny	90	stale	92	coarse
74	93		92		91	flat, oily
75	91	malty	92	feed	92	briny
76	93	sl feed	91	sl stale	91	feed, stale
77	93		89	cheesy	91	woody
78	92	stale	91	sl stale	92	sl stale

TABLE 3

Flavor criticisms used in scoring butter

Flavor criticism	Fresh score	Holding test 7 days—70° F.	Held 1 month 40° F.
Number of lots scored	78	78	78
No criticisms	20	16	15
slight old cream or old cream	8	0	3
slight acidy, acidy or coarse	14	13	11
briny	6	4	9
slight feed, feed or weedy	5	1	1
neutralizer	3	0	1
slight unclean or unclean	3	4	1
Cooked, heated	4	0	0
tallowy	0	4	0
slight storage or storage	0	5	9
slight stale or stale	2	15	18
slight cheesy, cheesy or fruity	0	8	1
utensil	4	0	0
slight rancid or rancid	0	5	3
oily, stale	1	2	4
malty	3	0	0
moldy	0	1	1
burnt	4	0	0
fishy	0	0	1
bitter	3	1	0
flat	3	2	0
woody	0	0	2
metallic	3	1	0

lower temperature of 40° F. there was very little evidence of bacterial activity which could be directly associated with flavor deterioration although large numbers of proteolytic bacteria were occasionally found after one month at 40° F. Lipolytic bacteria were absent except in a few lots in which small numbers, usually less than 1000 per ml. were found by the plate method.

Yeast and mold counts on the fresh butter failed to show any relation to the keeping quality of the butter in these trials. This is in agreement with the statements of numerous investigators working on this problem. The number of yeasts was generally high in the butter held at room temperature for 7 days but no correlation with the flavor deterioration could be noted.

CONCLUSIONS

In conclusion, these results indicate that the holding test is useful and fairly accurate as a means of detecting butter of unstable handling quality. The chief factor influencing the reliability of the test appears to be the difference in activity of certain types of bacteria at the incubation temperatures and at lower temperatures.

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A NEW DILUENT FOR BOVINE SEMEN

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A desirable diluent for semen is one that will maintain fecundity of the spermatozoa for many days before it is used for breeding purposes.

Considerable experimental work with diluents versus no diluents for semen has been done in Europe and the United States. The use of egg yolk lecithin in a diluent for semen by Milovanov and Selivanova (2) in Russia, and the use of egg yolk by Phillips and Lardy (4) of Wisconsin has proved helpful in keeping sperm cells viable for some time. The work of Milovanov (2, 3) and Phillips and Lardy (4) suggested an investigation of a combination of gelatin, egg yolk, buffer salts and water, as a diluent material in the artificial insemination studies in progress at the Ohio Agricultural Experiment Station. It is believed that gelatin (Knox) tends to hold sperm inactive, assists in keeping the particles of the egg yolk and the sperm in suspension, supplies extra nutrients, and retards general contamination (bacteria and molds) during storage.

Preliminary work done at this station with the gelatin and the non-gelatin diluents has given encouraging results in favor of the gelatin. Two series of samples have been studied. In the first series 12 samples of bovine semen were diluted four times with a diluent containing 2.14 gm. of gelatin (Knox), 0.2 gm. of KH_2PO_4, 1.325 gm. of $Na_2HPO_4 \cdot 12\ H_2O$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The non-gelatin group consisting of five samples of bovine semen were diluted four times with a diluent containing 0.2 gm. of KH_2PO_4, 1.325 gm. of $Na_2HPO_4 \cdot 12\ H_2O$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The materials other than the egg yolk of both diluents were first dissolved in the water before adding the egg yolk. The range in pH was from 6.7 to 6.85. The vials containing the diluted samples were wrapped in cotton and placed in a refrigerator maintained at 4° to 6° C. Periodical examinations were made with a microscope after a drop of the diluted semen was placed on a glass slide and warmed to 37° C. The gelatin dilutions maintained some sperm motility for an average of 21½ days (range 18 to 30 days), whereas the non-gelatin mixture maintained some sperm life for an average of 14½ days (range 14 to 16 days).

The technique of handling the semen in the second series was as follows: Collection and dilution of the semen was carried out under sanitary conditions; diluted samples were gradually cooled from 30° to 5° C. for storage purposes; and periodical examinations were made of the stored samples with a compound microscope (×440) after a small portion was rediluted and

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gradually warmed from 5° to 37° C. The gelatin and non-gelatin diluents were the same as those used in the first series, except that different amounts of gelatin and $Na_2HPO_4 \cdot 12\ H_2O$ were used from time to time. The range in pH was from 6.45 to 6.9. The results are given in table 1.

TABLE 1

The effect of gelatin and no gelatin in a diluent upon motility of bovine spermatozoa in storage

Diluent contains	Number of samples	Per cent of sperm motile after 2 to 4 days	Average number of days when 50 per cent motility was observed	Average number of days when 25 per cent motility was observed	Average number of days when all cells were dead
Gelatin (Knox)	26	73 (57–83)*	12.5	17.5	26.5 (16–35)*
No gelatin	14	59 (33–80)*	8.3	14.5	26.0 (14–38)*

* Figures in parentheses represent range.

Maintaining motility in 50 per cent of the spermatozoa an average of four days longer than previously possible and keeping a few alive for 35 to 38 days is stimulating to future work.

According to the literature the previous record for keeping motile bovine sperm in a diluent following collection seems to be 12.5 to 13 days (1, 4).

Artificial breeding of a large number of cows with semen that has been diluted with these two diluents is now in progress.

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SOME OCULAR CHANGES AND DEFICIENCY MANIFESTATIONS IN MATURE COWS FED A RATION DEFICIENT IN VITAMIN A*

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In previous publications (1, 2, 3) a type of blindness has been described which occurred in calves fed low vitamin A rations. The blindness was associated with a constriction of the optic nerve, nyctalopia, and papilledema. The cause of the papilledema was later established as directly due to an increased intracranial pressure (4) in vitamin A deficiency. This type of blindness has never been reported as occurring in the mature bovine because, as previously explained (2, 3), the optic foramina are fully developed and calcified. However, papilledema and nyctalopia develop as well as certain other ocular changes. It is the purpose of this paper to report the ocular changes and deficiency manifestations where the mature bovine was fed a ration deficient in vitamin A.

EXPERIMENTAL

Mature cows were used in this experimental work. They were placed on the low carotene ration previously used with calves which consisted of, 36.0 per cent barley, 27.0 per cent rolled oats, 27.0 per cent wheat bran, 9.0 per cent linseed oil meal, and 1.0 per cent salt. This ration contained from 0.5 to 0.7 micrograms of carotene per gram so that the animals received 2 to 3 micrograms per pound of body weight from this source. Viosterol and sunshine were used as sources of vitamin D. Wood shavings were used as bedding.

Ophthalmoscopic observations were made at various intervals and the animals were tested for night blindness by attempting to run them into objects, and watching their behavior in dim light, a method similar to that used by Guilbert and Hart (5). Blood plasma carotene determinations were made at intervals by a method previously described (6). Carotene extractions on the hays and feeds used were made according to the modification by Peterson *et al.* (7) of the Guilbert method and the concentration of the extract determined by a photoelectric colorimeter.

Before proceeding further it would probably be of assistance to the reader to explain the first three figures. Figure 1 shows the normal bovine fundus. The nerve head or papilla is seen in the center; the tapetum lucidum which consists of the upper yellow part of the retina and the

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tapetum nigrum the lower dark part of the retina are seen surrounding the papilla. There are of course individual variations of the normal fundus in the outline, color and distinctness of the nerve head, the color of the tapetum lucidum arrangement of the vessels, etc. The tapetum nigrum is always of a dark shade. The color of the tapetum lucidum is affected by the amount of exposure to bright light. The normal yellow color as shown in figure 1 will become bleached after the animal has been exposed to bright light such as the sun. These figures were made from animals not exposed to bright light.

Figure 2 shows the mottled appearance of the tapetum nigrum. The tapetum lucidum is bleached and the nerve head shows a cottony white appearance, evidence of papilledema.

Figure 3 shows the mottled appearance of the tapetum lucidum and papilledema. The mottled condition as illustrated in figures 2 and 3 occurs only in the more mature animal or after about 18 months of age.

A1 was a 5-year-old grade Holstein cow which had been receiving a ration in which the sole source of vitamin A was yellow corn. This ration, while apparently adequate for maintenance, had not contained sufficient vitamin A for proper reproduction. The vitamin A reserve for this animal was therefore probably much less than for an animal which had been receiving hay. Further, she had been milking on this ration up to the time she was placed on this experiment. The eyes were normal when placed on the low vitamin A ration except for a narrow violaceous area, 1½ cm. in length, along the temporal vessels.

After 18 days on the low vitamin A ration there was definite nyctalopia, the nerve heads of both eyes were somewhat hazy in appearance, and the tapetum nigrum showed some slight mottling as illustrated in figure 2. By 53 days the margins of the nerve head were definitely indistinct, but not markedly edematous, the mottling of the tapetum nigrum had increased, and the tapetum lucidum was somewhat bleached. At 81 days alfalfa was added, supplying 14 micrograms of carotene per pound of body weight. At 116 days this cow was no longer night-blind and the plasma carotene had increased from 0.18 to 0.4 micrograms per milliliter. The alfalfa was eliminated from the ration at 133 days and at this time the animal got out and obtained a good fill of green grass so that the plasma carotene increased from 0.4 up to 0.9 micrograms per milliliter. This accounts for the relatively long time it took to develop nyctalopia again.

At 236 days this animal again became nyctalopic and the edema of the nerve heads increased so that there was a choking of 2 diopters in each eye. At 249 days carotene was again added in the form of alfalfa at the rate of 14 micrograms per pound of body weight and at 269 days she was no longer night-blind. At 291 days both nerve heads were still edematous but the mottled appearance of the tapetum nigrum had quite largely disappeared.

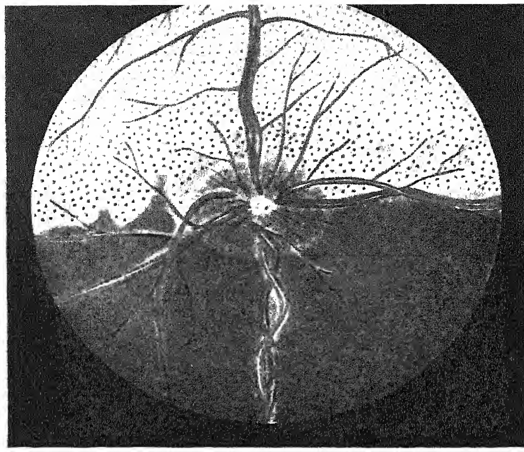


FIG. 1. Normal bovine fundus.

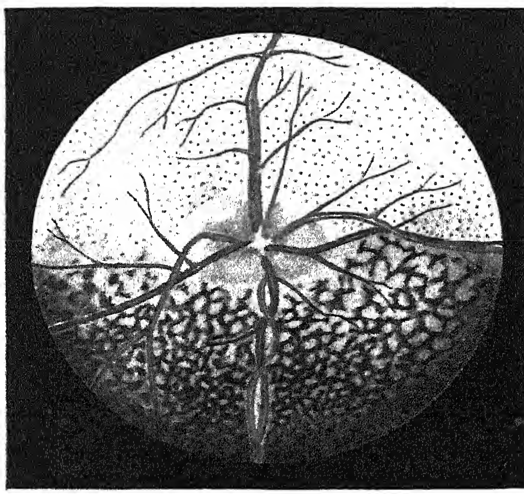
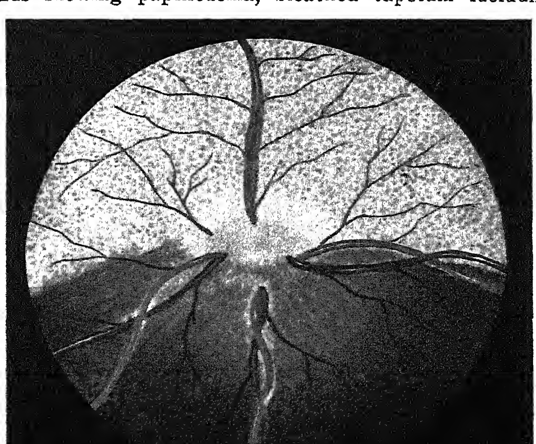


FIG. 2. Fundus showing papilledema, bleached tapetum lucidum and a mottled tapetum nigrum.

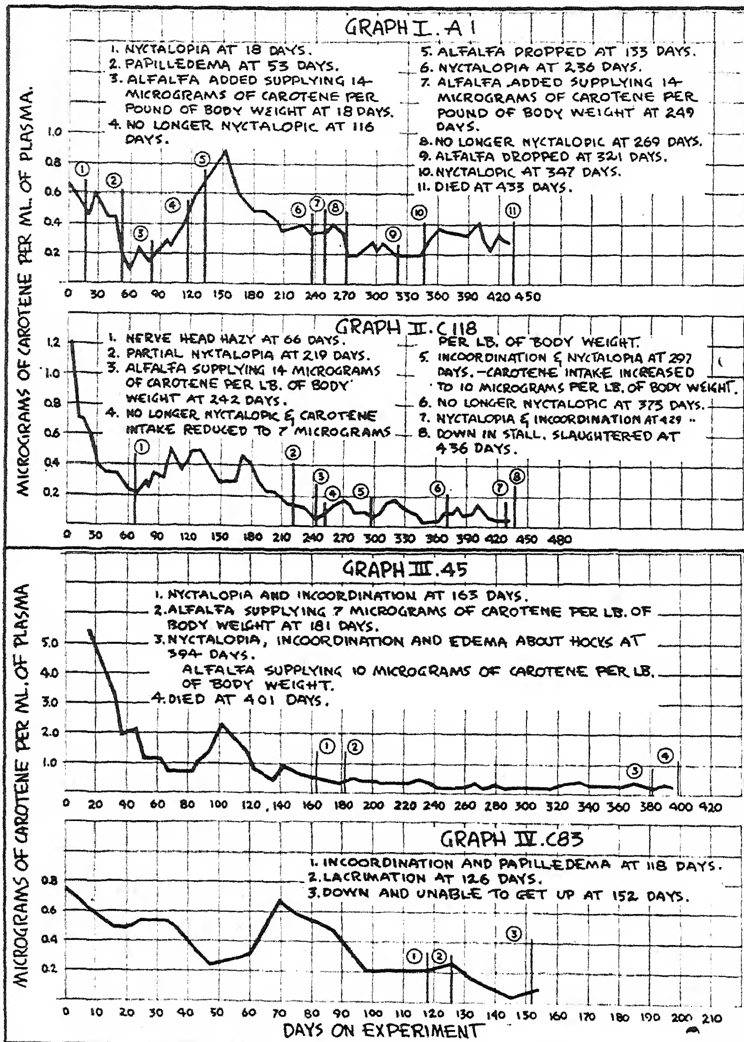


At 321 days the alfalfa was again eliminated from the ration. At 347 days she was again night-blind and by 421 days the edema had increased so that there was a choking of 3 diopters in both eyes and marked mottling of the tapetum nigrum. The cornea was slightly opaque and there was considerable lacrimation. At 411 days she was quite weak, showed marked incoordination and died at 433 days. The principal results along with the variations in level of blood plasma carotene are shown in graph I. Post-mortem examination revealed considerable pneumonia and other lesions associated with vitamin A deficiency which will be reported in a subsequent paper.

Cow C118 was a six-and-one-half-year-old Holstein cow which had been on a grain ration containing corn gluten and yellow corn as a source of vitamin A. This cow had been dry for a considerable period and calved about three weeks before being placed on the low vitamin A ration. This animal was dried up about three weeks after being placed on the low vitamin A ration so that she was milked for only about six weeks. Consequently, she probably had some storage when placed on the low carotene ration. The principal results are shown graphically in graph II.

Twenty-nine days after being placed on the low carotene ration, the tapetum nigrum of each eye showed definite mottling. At 66 days the papillae were quite hazy in appearance, but showed no elevation. At 95 days both nerve heads were hazy, but the margins could still be seen and there was no apparent elevation. She did not appear quite so active. At this time C118 got out of the pen at night and obtained some green material which probably delayed somewhat the later changes. At 165 days the tapetum lucidum was bleached and the tapetum nigrum quite mottled. At 219 days there was some indication of nyctalopia. At 230 days the animal showed considerable incoordination and a poor appetite. The nerve heads showed slight edema, but the margins were still discernible. There appeared to be little or no elevation. Alfalfa was added at 242 days to suply 14 micrograms of carotene per pound of body weight because of the extreme incoordination. At 262 days she was no longer night-blind, and the alfalfa intake was reduced to supply 7 micrograms of carotene per pound of body weight. At 276 days she no longer showed indications of incoordination and was fairly active although there was some edema in the rear legs. At 297 days she again manifested night-blindness, incoordination and had a rough appearance. At this time, the alfalfa was increased to 10 micrograms of carotene per pound of body weight. The incoordination largely disappeared at this level of intake but she remained partially nyctalopic till the 373rd day. The plasma carotene, however, remained exceedingly low at this level, and at 429 days nyctalopia and incoordination were again manifested. At 433 days the nerve head showed some edema, but the margins were still discernible. There was marked mottling of the tapetum nigrum and slight mottling of the lucidum as shown in figure 3. The tapetum lucidum was also

bleached. At 436 days the animal was quite weak, showed marked incoordination, got down in her stall and was unable to get up. The next day she was slaughtered in order to save the tissue for pathological study.



Cow 45 was a three-and-a-half-year-old Guernsey cow which had received a normal ration. She was placed on the low carotene ration the middle of June and had received some pasture so that the plasma carotene was quite high. After 163 days on the low carotene ration this animal showed nyctalopia, a slight bleaching of the tapetum lucidum, and was unsteady on her feet. The carotene in the blood plasma had decreased to 0.5 micrograms per

milliliter at this time as shown in graph III. After 172 days there was some yellowish discoloration just dorsal to the nerve heads. At 181 days alfalfa leaf meal was added to supply 7 micrograms of carotene per pound of body weight or one-half the minimum requirement. At this level she continued to show considerable incoordination and remained nyctalopic. At 349 days the tapetum lucidum was bleached somewhat but the nerve heads showed no evidence of papilledema. At 381 days the animal was unsteady on her feet, and there was considerable edema about the feet and legs. The carotene intake from the hay was then raised to 10 micrograms per pound of body weight.

At 394 days the tapetum lucidum of both eyes was entirely bleached and she walked slowly because of the edema about the feet. She died after 401 days on the low carotene ration without showing evidence of papilledema.

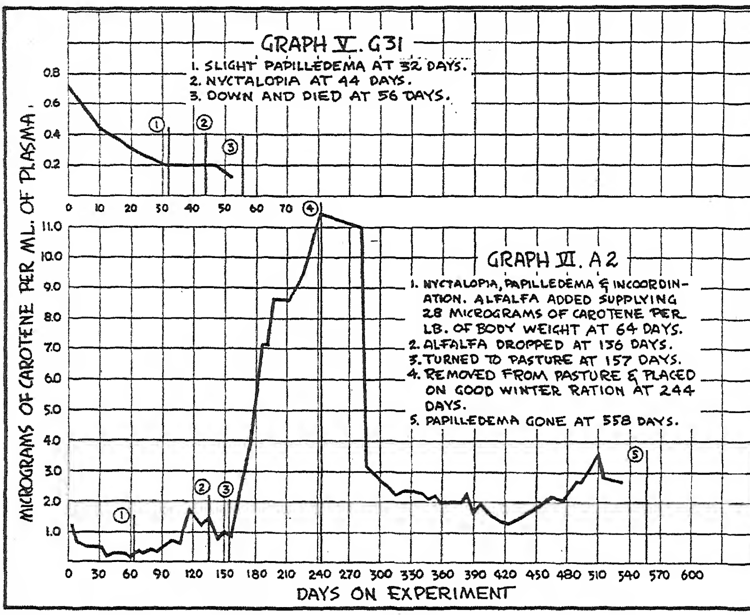
At post-mortem, aside from the changes due to vitamin A deficiency, the most notable phenomenon was the yellow color of the fat in the various parts of the body. A sample of the fat was weighed out, saponified with alcoholic KOH and extracted with petroleum ether. The petroleum ether was extracted with 92 per cent methyl alcohol to remove any xanthophylls. The extract showed a carotene content of 19 micrograms per gram. Further, this animal was in good condition and showed considerable deposition of fat in the mesentery. Post-mortem examination also showed the presence of pneumonia.

Cow C83 was an exceptionally fat seven-year-old Holstein which had previously been receiving a ration of skimmed milk, yellow corn, oats and viosterol. The ration had always been kept rather low in vitamin A during most of this animal's life. At the time this cow was placed on the deficient ration no routine ophthalmoscopic examinations were being made, nor was she tested for night blindness.

After 118 days on the low carotene ration marked incoordination was noted and examination of the eyes revealed an extreme papilledema. At 126 days excessive lacrimation was noted. At 145 days the appetite was poor and the plasma carotene had decreased to a 0.05 level as illustrated in graph IV. At 152 days she got down and was unable to get up. It was necessary to sacrifice the animal two days later because she could not get into position to eat.

G31 was a seven-year-old cow which had previously been receiving a ration in which the sole source of vitamin A was yellow corn. She was in excellent condition when placed on the low carotene ration and the eyes appeared normal. Thirty-two days after being placed on the low carotene ration the nerve heads were blurred in appearance. There was one diopter of papillary edema at 44 days in the right eye and 2 diopters in the left and the nerve head showed considerable vascularity. At this time she was also nyctalopic and walked rather slowly and stiffly. At 52 days there were 2

diopters choking in each eye. At this time she got down in the yard and was unable to get up alone. At 56 days she got down in the stall and was unable to get up. She was removed to a box stall and carotene in oil and linseed meal was administered by stomach tube. Cod liver oil was also given subcutaneously. She never regained her feet, however, and died during the night of tympanites. These observations as correlated with the level of blood plasma carotene are shown in graph V.



A2 was a five-year-old Holstein cow and had been receiving a grain mixture containing yellow corn as the only source of vitamin A. Ophthalmoscopic observations were not made on this animal until 64 days after she had been placed on the low carotene ration. At this time there was a choking of 2 diopters in the right eye and 4 diopters in the left and the nerve margins were entirely covered with the edema. She also showed considerable incoordination at this time and there was some suspicion that she was night-blind. The plasma carotene had decreased to 0.15 micrograms per milliliter. At this time alfalfa supplying 28 micrograms of carotene per pound of body weight was added to the ration. The alfalfa was dropped from the ration at 136 days. At 157 days the eye conditions remained the same and she was turned to pasture for the purpose of noting how long it would take these changes to clear up. It will be noted that the plasma carotene increased with this change as shown in graph VI. At 244 days she was removed from pasture and placed on a ration of alfalfa hay and corn silage which was followed by a rapid decline in plasma carotene. By 273 days the edema of

the nerve head had receded some and the nerve fibers could be seen.[1] At 341 days the nerve head had lost most of the cottony white color associated with the edema and had taken on a darker color which was more nearly normal. By 422 days the right eye was about normal but the margins of the left eye were still somewhat indistinct. By 558 days no edema could be seen in either eye and the nerve margins were fairly distinct. The nerve head, however, was still somewhat elevated since the readings were one diopter in the right eye and 2 diopters in the left. This was probably a residual effect of the long continued papilledema.

DISCUSSION

The results obtained with the animals of this experiment show that none developed the permanent type of blindness due to constriction of the optic nerve such as occurs in calves (1, 2, 3) on vitamin A deficient rations. Several of these animals were permitted to develop extreme deficiency symptoms yet never developed the blindness. Insofar as the author is aware, blindness has never been reported as developing in a mature bovine due to a constriction of the optic nerve associated with vitamin A deficiency. The explanation of this observation as previously set forth (2, 3) appears to be due to the fact the bony optic canal grows in length from one-fourth inch in a young calf to about one and a half inches in a mature animal. In vitamin A deficiency in a calf the normal growth processes are affected in such a manner as to cause a stenosis of the bony canal with a consequent constriction of the optic nerve (1, 2, 3). Wolbach and Bessey (8) have noted an overgrowth of the central nervous system in vitamin A deficiency in young rats. They noted the presence of herniations of the cerebrum, cerebellum and posterior colliculus with changes in the contours of the fossae of the floor of the skull due to bone resorption. If such an overgrowth of the central nervous system takes place in the bovine species it could easily account for the increased intracranial pressure reported from this station (4).

The results likewise show that papilledema does not develop as readily in mature animals as in young calves. Animal 45 did not develop evidence of papilledema while the nerve head of C118 showed only a slight hazy appearance. Usually the papilledema did not develop until considerable incoordination was present. Unpublished data indicate that the differences are probably explained by individual and age variations of intraocular tension. It would seem that a higher intracranial pressure would be necessary to overcome a high intraocular tension than a low one in order to permit the development of papilledema.

Besides the presence of papilledema in cows fed vitamin A deficient

[1] Papillary edema existing for any length of time results in secondary atrophy (post-papillitic atrophy).

rations, certain other changes were observed with the ophthalmoscope. These consisted of a mottled appearance of the tapetum nigrum and occasionally a mottled appearance of the tapetum lucidum. These two alterations are illustrated in figures 2 and 3 and may be compared with the normal fundus shown in figure 1. Both these conditions were cleared up by administration of some source of vitamin A. Usually the changes were more easily observed and were more marked in the nigrum than in the lucidum. The micropathological alterations of the retina associated with these changes have not been investigated.

The papilledema of animal A2 took considerable time to recede even though she was turned to pasture or kept on good winter feed. The observation was duplicated in another animal not considered in this report. In older calves the same was true but not to such an extent. It has also been noted in calves that the intracranial pressure takes considerable time to return to normal after the return of carotene to the ration (4).

It is interesting to note that animal 45, a Guernsey, had a very yellow fat at autopsy. The pigment was epiphasic between petroleum ether and 92 per cent methyl alcohol so that it was most likely carotene. These results seem paradoxical in view of the fact that the animal showed marked symptoms of vitamin A deficiency. One must conclude that the animal was unable to draw extensively from this store of carotene.

Another interesting observation was that the more flesh the animal carried at the time the deficiency started to show up, the quicker the animal succumbed to the deficiency. Warm weather also seemed to be hard on the deficient cows. C83 and G31 were in exceptionally good flesh and were able to withstand the effects of the deficiency for only a short time. On the other hand C118 and A1 which were poor when the deficiency symptoms were first observed seemed to withstand the deficiency much better. C83 and G31 both got down in the stall and were unable to raise their heads. It is thought that this acute condition is due in part to an abnormally high intracranial pressure. In another experiment the intracranial pressure of a young male on the deficient ration was found to be equal to 500 millimeters of saline while the normal is about 100 millimeters. This animal was just able to get up and draining the spinal fluid gave a short period of relief.

In calves when the level of plasma carotene had decreased to about 0.13 micrograms per milliliter nyctalopia and papilledema and other evidences of vitamin A deficiency began to appear (9). In mature cows this level would appear to be somewhat higher. Animal 45 showed evidence of nyctalopia at 0.5 micrograms level so that a range of 0.2 to 0.5 level should be considered. Davis and Madsen (10) reported a level of 0.25 for heifers of the Shorthorn and Hereford breeds.

From the results obtained with animals 45 and C118 it would appear that an intake of 9 to 12 micrograms of carotene per pound of body weight was

not sufficient to prevent the development of symptoms of vitamin A deficiency. This intake was made up of the carotene fed in the alfalfa meal at the rate of 7 to 10 micrograms and the 2 to 3 micrograms present in the basal ration per pound of body weight. A total intake of 16 micrograms, however, appeared to be sufficient as shown by the results for A1 and C118. These observations agree with our previous observations (9) and those of Guilbert and Hart (5) who found that an intake of about 30 micrograms per kilo was necessary to prevent nyctalopia. However, it seems questionable whether this intake is the physiological minimum as stated by Guilbert and Hart since it is not sufficient for proper reproduction, or to prevent the development of an increased intracranial pressure in calves as shown by unpublished data from this station.

It will be noted in this paper that no cases of xerophthalmia are recorded even though extreme vitamin A deficiency was permitted to develop. In some cases considerable lacrimation and some clouding of the cornea were noted. The absence of xerophthalmia was confusing in the early work at this station on vitamin A deficiency and led to considerable doubt as to whether the deficiency seen was actually due to lack of vitamin A (1). However, the explanation of the apparent discrepancy probably lies in the environmental conditions under which these experiments were conducted. The eyes of the animals during the deficiency period were probably not subjected to the presence of large amounts of abrasive dust particles and possibly the proper type of bacteria.

SUMMARY

1. Mature cows on a vitamin A deficient ration failed to develop blindness due to constriction of the optic nerve such as has been reported in calves.
2. A definite papilledema failed to develop in two cows out of six in these experiments. Once the papilledema develops it takes considerable time for it to recede.
3. Mature cows did develop nyctalopia, incoordination, and an edema of the legs on the A deficient ration.
4. The tapetum nigrum and lucidum developed a mottled appearance.
5. When the plasma carotene values receded to a 0.2 to 0.5 microgram level deficiency symptoms usually followed in a short period of time.
6. The fat of a Guernsey cow which died with symptoms of vitamin A deficiency showed the presence of a pigment which was most likely carotene since it was epiphasic between petroleum ether and 92 per cent methyl alcohol.

The author wishes to express his appreciation to Dr. J. O. Wetzel of Lansing for his criticisms in preparing this paper.

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Announcement

Translations of a number of Danish and Swedish articles of interest to readers of the JOURNAL OF DAIRY SCIENCE have been completed in a W.P.A. project sponsored by the University of Minnesota. These translators assigned to W.P.A. Official Project No. 65–1–71–140, Sub-Project No. 484, have been supervised by Dr. Harold Macy. Copies of these translations are available in the Office of the American Documentation Society, 2101 Constitution Avenue, Washington, D. C.

Translations of the following Danish dairy articles are now available.

Smørrets Vandindhold og Saltning (Water content and salting of butter). H. Hendemann. 15de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med Silkeborg Stassano apparat Model 1937. (Experiments with Silkeborg Stassano apparatus, Model 1937). Joho. Jensen and others and Sv. Horning. 16de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med "Spirala" til Varmebehandlung af Konsummaelk. (Experiments with "Spirala" for heat-treatment of consumers' milk). H. Jörgensen. 17de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

Forsøg med "A.P.V." Apparat til Varmebehandling af Konsummaelk. (Experiments with the "A.P.V." apparatus for heat-treatment of consumers' milk). A. Petersen and K. Rasmussen. 18de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

Forsøg med Pladeapparat "Kolding" Type B.P.K. til Varmebehandling of Konsummälk. (Experiments with the plate apparatus "Kolding," type B.P.K. for heat treatment of consumers' milk.) N. Kjärgaard-Jensen. 20de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–26. 1939.

Af prøvning af Victoria-Kubus Kaerneaelter. (Testing of the Victoria-Kubus churn-worker.) N. Kjärgaard-Jensen. 22de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–21. 1939.

Forsøg med Victoria-Kubus Kaerneaelter. (Experiments with the Victoria-Kubus churn-worker.) N. Kjärgaard-Jensen. 24de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–28. 1939.

Translation of the following Swedish article is also available.

Inverkan av vissa Konserveringsmedel på Mögel—och Jastsvampar från Ost. (Effect of certain preservatives on moulds and yeasts from cheese.) K. E. Thome. Meddeland No. 3 fran Statens Mejeriförsök Särtryck ur Arsskrift for Alnarps lant-bruksmejeri-och trödgårds-institute, pp. 1–20. 1939.

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Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION

658. The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria.

JOHN C. GAREY, EDWIN M. FOSTER AND WILLIAM C. FRAZER, Department of Agricultural Bacteriology, University of Wisconsin.

Brick cheese was manufactured by the conventional method with *Streptococcus lactis* and *Streptococcus thermophilus* starters used singly and in different combinations. The growth and activity of the starters were followed by bacteriological, chemical and physical methods.

When 0.6 per cent of *Str. lactis* starter was used alone and the curd cooked to 106° F., the development of the lactic streptococcus was very slow until the third or fourth hour after dipping; thereafter the numbers increased rapidly and reached their maximum at one to two days. If the curd was cooked at 112° F., the growth of the *Str. lactis* bacteria was decreased as evidenced by a slower rate of multiplication and a lower maximum number. Because of the lack of activity of the lactic streptococcus until the latter part of the draining, it was necessary to dip the curd in a relatively dry condition otherwise the cheese would retain too much moisture and those defects characteristic of an acid cheese would develop.

When 0.6 per cent *Str. thermophilus* starter was used alone and the curd cooked to 106° F., growth of the starter bacteria was most rapid during the cooking and the first three hours after dipping; thereafter the rate of multiplication decreased sharply because of the unfavorably low temperature in the cheese. A cooking temperature of 112° F., in comparison with 106° F., increased the growth and activity of the thermophilic streptococcus. When *Str. thermophilus* starter was used alone, all the lactose was not fermented in the cheese. This was evident from the high pH at one day, the later development of *Str. lactis* in large numbers and the development of undesirable bacteria which produced gassiness and fermented flavors in the cheese.

Alone, neither of the starters produced a Brick cheese of satisfactory quality. The cheese manufactured with *Str. lactis* starter developed a sour flavor and a short and crumbly body, that with *Str. thermophilus*, a fermented flavor and a very open texture. Of the different combinations of starters tried (cooking temperature—106° F.), a mixture of 0.3 per cent each of *Str. lactis* and *Str. thermophilus* produced a Brick cheese of more desirable quality than that with the other combinations.

If the moisture was higher than 42 per cent after salting, it was a practical guarantee of an acid or sour cheese with characteristic defect. This

meant that the moisture of the ripened cheese had to be 2 to 4 per cent lower than the legal limit in order to produce a desirable Brick cheese.

659. **Lipolytic and Proteolytic Activities of Various Penicillia.** C. JENSEN, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

Studies were made of the lipolytic and proteolytic activities of the penicillia employed in the ripening of blue veined cheeses. The report deals with a study of 23 strains of penicillia including one *P. chrysogenum,* 3 *P. gorgonzola,* 15 *P. roqueforti,* one *P. stilton* and 3 unidentified strains.

There was considerable variation in the lipolytic activities of various penicillia on butterfat and cottonseed oil, as determined by the nile blue sulfate technique; the intensity and uniformity of lipolysis of the cultures ranged from nonlipolytic to very pronounced lipolytic.

There was considerable variation in the lipolytic activities of various penicillia on different triglycerides according to the nile blue sulfate technique. Only a few cultures hydrolyzed tripropionin, while all readily hydrolyzed tributyrin and trivalerin. As the molecular weights of the triglycerides increased, variation in lipolytic activities became more conspicuous. Some cultures showed gradual declines in their lipolytic activities, whereas others declined sharply on the triglycerides beginning with tricaproin.

There was considerable variation in the toxic effect of different triglycerides on various penicillia. In general, the triglycerides that exhibted the most pronounced toxicity, in declining order of their effect, were tripropionin, tributyrin, trivalerin, tricaprin, trilaurin, trimyristin and tripalmitin. The least toxic were triheptylin, tricaproin and triolein.

There was considerable variation in the proteolytic activities of various penicillia as determined by the acidified milk agar and the carbon dioxide techniques. There was a general agreement between the results obtained with the two techniques.

The rates of growth and of proteolysis of certain penicillia were affected by different growth conditions. The cultures grew more slowly but showed greater proteolytic activities in air at 28° C. than at 19° or 12° C.; the cultures were somewhat retarded in growth but proteolysis was unaffected when grown at 28° C. in an atmosphere in which 10 per cent of the air had been replaced by carbon dioxide; culture growth and proteolysis at 28° C. were almost stopped in an atmosphere saturated with carbon dioxide; growth usually was unaffected but proteolysis was accelerated at 28° C. in an atmosphere consisting for the most part of nitrogen.

660. **A Test for the Protein Stability of Milk.** ARNOLD B. STORRS, American Seal-Kap Corporation, Long Island City, N. Y.

A test for protein stability which requires a minimum of equipment, reagents and technical skill is described. Increasing amounts of N/10 HCl

are added to 10-ml. portions of the milk to be tested. The mixtures are placed in a boiling water bath for 10 minutes and then examined for coagulation. The stability number is equivalent to $100 \times$ the ml. of HCl required to produce coagulation under the conditions of the test.

The average stability number of untreated fresh milk has been found to be about 60 to 70 as indicated by the test. Pasteurization tends to increase the protein stability of milk while copper contamination tends to lower the stability.

661. **A Method for the Estimation of Nicotinic Acid in Milk.** E. A. Bailey, Jr., W. J. Dann, G. Howard Satterfield, and C. D. Grinnells, Duke University and North Carolina State College of Agriculture.

A chemical method for the estimation of nicotinic acid, the pellagra-preventive factor, in milk is reported. The essentials of the method are: Acid hydrolysis, removal of interfering salts and colored material by treatment with Lloyd's reagent and lead hydroxide, and development of a colored complex by treatment with cyanogen bromide and metol (p-methylaminophenol sulfate). The reproducibility of results and the recovery of added nicotinic acid by this method are found to be satisfactory.

The analysis of milk from Ayrshire cows during the month of January 1941, shows the normal nicotinic acid content of the milk from these cows during this period to have been 1.46 micrograms per ml.

The low value obtained for the nicotinic acid content of milk is of interest in view of the fact that milk has long been considered of great importance in pellagra-preventive diets and has been shown to have pellagra-preventive value. The possibility is suggested that when considerable quantities of milk are included in the diet, the intestinal flora are so altered that a significant amount of nicotinic acid is synthesized by the intestinal microorganisms.

662. **The Effect of the Administration of Shark Liver Oil on the Butter Fat and Milk Production of Cows.** H. J. Deuel, Jr., Department of Biochemistry, University of Southern California Medical School, Los Angeles.

Shark liver oil was administered to six Guernsey cows in amounts of 30 cc. daily (700,000 I.U. of vitamin A) and a comparison in milk production was made with that of six other cows receiving the same basal diet without the supplement. All cows received the same basal diet which included large amounts of fresh-cut alfalfa. In a five-week preliminary period, the average milk production was practically identical in the two groups. With the feeding of the shark liver oil, an immediate rise in milk production of approximately 10 per cent over the control level was noted which continued

for 11 weeks; this gradually increased to a value of over 20 per cent by the 16th week of the test. An increase in butter fat given by the cows receiving the oil supplement over that of the control animals varied during this period between 507 and 794 grams per week per cow. These alterations are not ascribable to season, lactation cycle, or to food and are believed to be caused by the administration of the shark liver oil.

663. Age, Live Weight and Milk-Energy Yield—A Correction. W. L. Gaines, C. S. Rhode and J. G. Cash, University of Illinois.

This correction removes certain systematic errors in the live weight estimates of a previous paper (this journal Oct., 1940). The most important change thereby effected is that the new (more correct) live weight data show that within herd milk-energy yield is proportional to the 1.02 power of live weight in the Holstein records; and proportional to the 0.98 power of live weight in the Jersey records. Live weight is measured within the first 31 days after calving and yield is for the first 8 months of the lactation.

BACTERIOLOGY

664. The Bacteriological Analysis of Creamery Waters. H. Wolochow, Science Service, Dept. of Agr., Ottawa, Ont.; H. R. Thornton, Univ. Alberta, Edmonton, Alberta, and E. G. Wood, Science Service, Dept. of Agr., Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 23. 1941.

Creameries in many parts of Canada have experienced very serious trouble in the past few years from butter deteriorations caused by the use of contaminated water supplies, although in many cases these waters were of potable standard. Eighty-five samples of water from 37 Alberta creameries were examined for specific types of organisms. A surprising number of the waters contained large numbers of bacteria capable of growth at 10° to 15° C. Many of the bacteria were proteolytic according to the criteria applied. Further investigation may indicate the necessity of stricter bactericidal treatment of all creamery waters. O.F.G.

665. Burri Technique Discussed. H. H. Weiser, Ohio State Univ., Columbus, Ohio. Amer. Milk Rev., *2,* No. 7: 157–158. July, 1940.

The Burri agar slant method offers an inexpensive, rapid, simple procedure for determining relative numbers of bacteria in milk, and may be modified for use with butter, cheese, and ice cream. The original method has been considerably modified. Directions for making the test are given. The method gives better differentiation of colonies than the ordinary plate method because all colonies are on the surface; the count is lower because

clumps are not completely broken up; composition of the medium may be varied to suit the growth of particular types of bacteria; and it offers a good means for cultural examination of mastitis milk. P.S.L.

666. **Heat Resistant Bacteria.** A. C. Maack. Amer. Milk Rev., *3,* No. 1: 1–2. Jan., 1941.

Survival of heat resistant bacteria after pasteurization presents a serious problem in attempts to regulate plate counts of pasteurized milk. Both thermophilic and thermoduring bacteria compose the groups surviving, and both rod and spherical forms are found. Feed, bedding, and soil harbor thermophilic organisms and utensils and milking machines often are a source of thermoduric types. Milk from suspected farms may be plated to locate sources. Exposure to chlorine of 100 p.p.m. for 2 or 3 minutes is necessary to kill these organisms. Repasteurization is often a cause of trouble as is incomplete pasteurization of foam, and milk stone in vats is strongly suspected as acting to harbor bacteria of these strains. In controlling infection all utensils must be sterilized. Infections are more common during cold weather when carelessness in cooling is more apt to occur. P.S.L.

667. **Is Zero To Be the Limit.** M. E. Parker. Amer. Milk Rev., *3,* No. 6: 128–130. June, 1941.

In this article the author has reviewed action of the Coordinating Committee on Standard Methods of the American Public Health Association to better the method of making plate counts of milk. The author suggests that emphasis be placed on qualitative rather than quantitative methods for evaluating quality of milk. He believes that such quantitative emphasis will result in lowered palatability, that it will gradually reach a point where cost would not be justified by the safety attained, and that the plate count for evaluating quality is futile and confusing. Adoption of cultural media designed to develop the maximum number of bacteria does not give a true picture of the numbers of bacteria in milk causing spoilage. It will cause reduction of counts by increasing the care given to milk production but will not necessarily increase quality. Lowering bacterial counts has increased susceptibility to oxidized flavor in milk and surface taint in butter, due to the upsetting of the balance in the normal bacterial flora. If the goal is zero it means reduction in quality with consequent reduction in consumption. P.S.L.

BREEDING

668. **Eine volle Laktationsperiode umfassende Amidfutterungsversche mit eineiigen Rinderzwillingen.** J. Schmidt and J. Kleisch, Univ. Berlin. Züchtungskunde, *15,* No. 16: 169–174.

A pair of identical twin heifers were fed through a whole lactation period

with one of them receiving in the form of amide nitrogen almost half of the protein judged necessary by present feeding standards. The fat percentage was identical and the production for the heifer receiving the amide nitrogen was 3755 kg. of milk and 126.5 kg. of fat, as compared with 3936 kg. of milk and 132.6 kg. of fat for the other heifer. No differences in the general health of the animals were observed. J.L.L.

669. **Vergleichende Untersuchungen über den Zusammenhang zwischen Alter und Leistung bei verschiedenen Rinderrassen, durchgeführt an Kühen des Deutschen Rinderleistungsbuches (RL).** J. Lange, Univ. Berlin. Züchtungskunde, *16,* No. 4: 123–126. 1941.

Whether dairy breeds differ in the regression of milk and fat production on age was studied on data from the German "Rinderleistungsbuch." These data correspond somewhat to the data from the Herd Improvement Registry testing in the United States, except that the German data are reported by association testing year instead of lactations, and the cows are selected cows. The lifetime records of 2,113 cows of the lowland races and 452 cows of the highland breeds of Germany were studied. Each of these cows had at least 7 years of records. By this restriction it was thought that the effects of selection on the age curve would be avoided, but the possibility of the opposite error arising because of the imperfect repeatability of the records on which past selection may have been based is not considered.

In general the breed-to-breed differences were small and it is doubtful whether they were statistically significant. The middle German red breed did not decline as rapidly after maturity as the others and this is attributed to innate resistance and hardiness. The Oldenburger division of the black and white lowland cattle showed an earlier rise to the peak of production and a quicker decline afterwards, which is attributed to the influence of some Shorthorn blood introduced long ago. The Angler breed showed less extreme changes than the black and white lowland cattle. These differences

Year on test	Milk quantity		Fat (per cent)	Total fat	
	Kg.	Relative		Kg.	Relative
2nd	4111	76.3	3.57	146.7	77.0
3rd	4632	85.8	3.59	166.1	87.2
4th	5082	94.3	3.57	181.5	95.3
5th	5268	97.8	3.57	188.0	98.7
6th	5373	99.7	3.55	190.5	100.0
7th	5388	100.0	3.53	190.0	99.7
8th	5296	98.2	3.54	187.6	98.5
9th	5212	96.2	3.51	182.8	96.0
10th	5109	94.8	3.52	179.8	94.4
11th	5049	93.7	3.48	175.5	92.1
12th	5150	95.6	3.38	174.3	91.5

were slight and for practical purposes it may almost be said that there were no breed differences in the relative changes of production with age.

The change in fat percentage with advancing age was small and no breed differences were found in the shape of that curve. The maximum production of total fat was reached in the sixth association year which would be at an age of about 8 or 9 years. The following table shows the averages by year on test. The year the cow first came on test was omitted, since that would be fragmentary in nearly all cases.

J.L.L.

BUTTER

670. The Yeast and Mold Service in Relation to Quality Improvement. W. H. Sproule, Dairy Dept., Ontario Agr. College, Guelph, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 1: 50. 1941.

Yeast and mold counts have been recognized for a considerable period of time as playing an important part in laboratory control of butter quality. While butter containing numerous yeasts and molds might give good commercial satisfaction at times, as shown by some of the work accomplished, nevertheless, the larger creameries recognized that butter with a low yeast and mold content was a better risk for storage purposes than butter made in a less sanitary way. In reviewing the progress made during 8 years, D. B. Shutt reported marked improvement as the work progressed. In 1928, 34.8 per cent of submitted samples had counts of 10 yeasts, or less, per cc. as compared with only 1.2 per cent in 1921. The mold counts also showed improvement; 61.8 per cent of the samples showed counts of 10 or less in 1921, whereas, in 1928, 89.9 per cent fell in this class. The butter laboratory is at present making analyses for yeast and mold on samples submitted by creameries which have applied for the service. O.F.G.

671. The Experimental Error in the Plate Count Examination of Butter. E. G. Pont, Dept. of Agriculture, Sydney, Australia. Jour. Dairy Res. *12*, No. 1: 24–34. 1941.

In an investigation into the experimental error of the plate count of butter, 154 boxes of butter were examined by plating in triplicate, in a dilution of 1/500, each of three 1-gram samples per box. The means of the triplicate counts on any one sample, judged by conformity to the Poisson distribution, were considered to give reasonably satisfactory estimates of the bacterial content of the samples. The between-sample variability was shown by transforming the counts to logarithms and calculating the coefficient of variation in respect of each box. In the distribution of the coefficients approximately 50 per cent was found to lie on either side of a 4 per cent level, while 10 per cent gave values higher than 14 per cent.

In a further study 12 boxes of butter were selected for quality and uni-

formity and data were secured from the examination of 7 1-ounce samples selected at random from each box. Using the method of analysis of variance, the results indicate that the estimates of within sample variance obtained would be regarded as estimates of a common variance. High significant differences were found, however, among the between-sample mean squares and the variability was found to be excessive in six of the twelve boxes examined.

The author points out that owing to the excessive between-sample variance found, the result of a single plating used as an index of the mean bacterial population of a box of butter may be quite inaccurate. It is only when a high estimate is encountered (*e.g.*, several hundred thousand or more per gram) that any real degree of significance can be attached to the result. Errors arising from technique would be unlikely to influence a normally low count to this extent. The occurrence of butter giving rise to such a count, even though it appeared only in parts of a box or a churning, would, from the standpoint of quality control, indicate the need for remedial measures. S.T.C.

672. Facts to Know about Packaging Butter. L. C. THOMSEN. Amer. Butter Rev., *2*, No. 4: 114, 116, 128. 1940.

Figures from several localities in this country show surprisingly large quantities of bulk butter are still sold to the public. Most of this comes to the dealer in spruce tubs, notorious for their tendency to transmit wood flavor to butter. Paraffin retards, but does not prevent, absorption of these flavors. Anti-oxidants applied to parchment liners, according to preliminary work, do not prevent absorption. The casein-formalin treatment, while perhaps effective, has not been looked upon favorably in this country. Avoidance of air pockets in packing butter reduces aerobic conditions necessary for the growth of bacteria causing spoilage; careless storage conditions for sterile parchment may result in mold and yeast contamination; and treatment of parchment circles and liners with salt brine and calcium propionate further reduces mold. The use of the latter alone in 10 per cent solution has been reported as responsible for surface mottling of butter.

The author predicts as the next great advance in packaging butter, a continuous churn with packaging following directly from the churn. Wholesaler's demands for amount of overweight per 64 pound tub, he states, varies from 6 to 12 ounces. From two to fifteen per cent of butter reaches the market underweight. On the average when butter is cut the overweight per uncartoned pound print is $\frac{1}{4}$ ounce. Losses in cutting and in overweight may amount to $\frac{3}{4}$ to one pound per 64 pound tub. Overweights given per pound of butter amounts to $\frac{2}{3}$ pounds per hundredweight of butter cut. Shrinkage of pound prints of dry wrapped butter is about $\frac{1}{16}$ ounce more than for prints wet wrapped. Machine wrapping and cartoning costs for

one pound prints vary from 0.76 to 1.35 cents per pound; and for quarter pounds, from 1.0 to 1.36 cents per pound. The latter, uncartoned, costs 0.75 cents per pound. P.S.L.

673. **Manufacture and Use of Butter Culture.** N. E. FABRICIUS. Amer. Butter Rev., *2*, No. 3: 74, 92. 1940.

The author reviews the arguments for and against the use of starter, the organisms responsible for flavor in starter, the products formed by starter bacteria, and the problems involved in the successful carrying on of cultures. He recommends heavy inoculation of cultures, one pint or quart per 10 gallons of milk; the addition of two ounces of citric acid crystals dissolved in ½ pint of hot water per 10 gallons of milk; ripening the culture to a higher degree of acidity than ordinarily practiced, and using taste or the creatine test rather than acidity test for determining the degree of ripeness; quick cooling with vigorous stirring; and cooling to a low temperature to prevent reducing diacetyl to flavorless, 2, 3-butylene glycol. As between addition of starter to cream at 70° F. allowing to grow a few hours and cooling, or addition of starter to cooled cream and holding 8 to 12 hours before churning, the author prefers the latter procedure because a greater quantity of the acetylmethyl-carbinol is oxidated to diacetyl. P.S.L.

674. **Testing Cream for Mold Mycelia.** C. H. PARSONS. Amer. Butter Rev., *2*, No. 11: 382–384. 1940.

The details of procedure for the Parsons Visual Mold Test together with a list of required equipment is given in this article. The author suggests classification of cream into four grades according to mold content. Being both inexpensive and simple the test is recommended as a routine measure in cream stations and butter plants. P.S.L.

675. **On the Receiving Line.** V. SCHWARTZKOPF. Amer. Butter Rev., *2*, No. 12: 406, 408, 410, 412. 1940.

Quality of cream will be maintained if it is handled promptly at the plant, graded and churned by grade, contamination avoided, metallic taint prevented through use of well-tinned cans, and return to the farm of clean, dry cans. For cleaning cans the use of soft water will prevent reduction of washing machine efficiency by preventing formation of sludge which clogs pipes, tank and pumps. Washing powder that produces no bacteria harboring sludge is best. It must be free rinsing and capable of removing all deposits quickly and completely and be a good water softener if hard water is used. Temperature of water should be 140°–145° F. and alkalinity, 0.3 per cent or less. After washing, the can should be rinsed in clean hot water at 190° to 200° F., although the rinse, if hard water, leaves a film of mineral

on the can. Use of softended water eliminates this difficulty. After steam or hot water sanitization it is desirable to dry the can with air heated to 250° F. or higher. Such procedure seldom gives an absolutely sterile can. Sterilizers such as 1:100 Zephrin are efficient but costly. Sterilization by light gives little promise. Straight-side cans are more easily cleaned.

P.S.L.

676. Counting Mold Mycelia in Butter. G. W. SHATTUCK, JR. Amer. Butter Rev., *3*, No. 1: 10, 12. 1941.

A complete outline of the method for counting mold mycelia is given. Recommendation is made that the microscope be standardized to cover a field 1.382 mm. in area and that no field is reported positive unless one filament or the combined length of the two longest filaments exceeds $\frac{1}{6}$ the diameter of the field.

Filters aid in picking out the filaments. Use of five per cent aqueous solution of crystal violet as a stain in preparing the sample outlines the mycelia sharply making possible their identification as single or broken filaments and making their measurement more conclusive. Efforts to simplify mold mycelia counting procedure have been disappointing. In case the count is low or high, the counting of 50 fields is satisfactory, but where the count is close to 60 per cent positive, the examination of 100 fields is preferable. A tolerance of 10 per cent difference in counting between technicians is allowable. Observation leads to the theory that length of the mold filament is correlated with age of cream. P.S.L.

677. Cream Grading and the Future of the Butter Industry. C. E. LACKNER, Dept. of Agr., Toronto, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 62. 1941.

The author points out that, although the fear of surplus Canadian butter is passed now, there is likely to be a surplus again when world conditions become normal. The quality of Ontario cheese has been good but a considerable portion of the butter has been of low grade due primarily to poor quality in the cream. Looking ahead, therefore, to normal market conditions when a surplus of butter can be expected it is essential that a sound cream-grading program be promulgated now. O.F.G.

678. Bacteria in Well-Waters. C. H. CASTELL AND E. H. GARRARD, Ontario Agricultural College, Guelph, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 18. 1941.

As pasteurization of cream and improved sanitation has decreased certain types of butter and cream spoilage, the importance of trouble from what appears to be minor sources has become more significant. One of the most important of these is water used by creameries and owing to the peculiar

characteristics of water bacteria, the milder and less salty butter is made, the more their activity will be noticed. Ten per cent of the samples examined were found unfit for human consumption, 30 per cent showed the presence of butyric acid forming anaerobic bacteria, a majority of the waters contained organisms which were oxidase-positive, and approximately 85 per cent contained organisms capable of growing at a temperature within 4 or 5 degrees of freezing and at the same time capable of decomposing fat and curd. Results suggested the presence of *Pseudomonas fragi* and *Pseudomonas fluorescens*. O.F.G.

679. **Factors Influencing Mold Mycelia in Cream.** P. R. ELLIKER, Purdue Univ. Natl. Butter and Cheese Jour., *32*, No. 7: 8. 1941.

The velvety, white growth commonly found on the surface of sour milk or cream is *Oospora lactis*, "milk mold." It gets into milk and, eventually, cream and butter through dust, dirt, manure and utensils. Some strains grow as low as 40° F., others as high as 100° F., but ideal temperatures approximate 75° F. It is destroyed by proper pasteurization. Growth of *Oospora lactis* is favored by a slightly acid reaction and is retarded or inhibited by high acidity, lack of air, presence of salt and possibly propionic acid or its salts. Its presence in butter with yeasts and other molds indicates unclean churns or equipment. It forms part of the surface flora of some cheese. Delivery of cream 3 times weekly eliminates the mold problem if clean utensils and separators are used and if cream is stored at 60° or lower. The mold content of gathered cream decreases as the fat increases from 30 to 50 per cent, probably because it is machine- rather than hand-separated but perhaps because rich cream may be a less favorable medium for mold growth. Frequent stirring of cream decreases the mold count on butter but may injure the quality of the butter because it encourages undesirable changes in the cream. A large surface area of cream in relation to amount of cream increases mold growth. Delays in neutralizing and pasteurizing cream permit mold growth. During the processing of cream and butter, mold filaments are broken. Although some of the mold may go into the buttermilk, still a high mold content in the cream causes a high mold content in butter. W.V.P.

680. **Safeguarding Butter Quality.** B. F. MCKIBBEN. Amer. Butter Rev., *2*, No. 3: 78, 92. 1940.

In a discussion of the thirty-five off flavors listed in the government score card for butter this writer emphasized the danger of transmitting lubricating oil flavors to cream from oil lubricated pumps, and cites the work of Turgasen as to the frequency of cheesy flavors from contaminated water. P.S.L.

681. **Recent Trends in Neutraliziation.** LEE H. MINOR. Amer. Butter Rev., *3,* No. 3: 90, 92. 1941.

Calcium in lime neutralizers has a greater affinity for casein or curd than for lactic acid and tends to increase viscosity of cream, produce lime flavor, and clog filters; sodium in neutralizers, while excellent for neutralizing lactic acid, has a tendency to create foam and has been accused as being responsible for soapy flavor. Combination of the two lessens the effect of each. With the vacuum process of pasteurization neutralizer may be added to the cold cream in the forewarmer, allowing it to act on the acid before pasteurization temperature is reached, reducing foaming and danger of saponification of fat. Dilution of the neutralizer with water is important, the amount increasing with the causticity of the alkali. P.S.L.

CHEESE

682. **"Phage" in Cheesemaking.** C. K. JOHNS, Div. Bacteriology and Dairy Res., Science Service, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 18. 1941.

Most makers of Cheddar cheese have had experience with slow acid development, an occurrence which is generally accepted as one of those things that cannot be explained and about which nothing can be done except to get a new starter. In New Zealand considerable use is made of single strain lactic acid bacteria starters. When such a starter becomes contaminated with bacteriophage the bacteria are destroyed practically 100 per cent and acid development ceases abruptly. In Canada, on the other hand, the starter is usually a mixture of several different strains or species of desirable bacteria. Since a phage generally attacks only one strain, or a few closely related strains, the result is usually a slow development of acid. The author, however, found a phage different from that reported in New Zealand which attacked every one of 10 different organisms found in the starter in use. Several outbreaks of slow acid development were found each of which disappeared when the plant and equipment had received a thorough house-cleaning and sterilizing treatment. O.F.G.

683. **The Drying of Cheese Whey and of Acid Casein Whey by the Roller Process.** R. WAITE, The Hannah Dairy Res. Inst., Kirkhill, Ayr. Jour. Dairy Res., *12,* No. 1: 71–77. 1941.

Sodium or potassium compounds were found to be unsuitable for neutralizing cheese whey prior to drying. Neutralization with calcium hydroxide gave a satisfactory product. Reduction of the acidity of hydrochloric acid casein whey to 0.18 per cent by the addition of calcium hydroxide allowed satisfactory neutralization. S.T.C.

684. Starter Cultures for Cheese Manufacture. Further Attempts to Eliminate Failures Due to Bacteriophage. H. R. Whitehead and G. J. E. Hunter, Dairy Res. Inst. (N.Z.), Palmerston North, New Zealand. Jour. Dairy Res., *12*, No. 1: 63–70. 1941.

Bacteriophages for lactic streptococci were found to occur in the atmosphere of commercial cheese factories. This was established in three ways: (a) aspiration of air, (b) exposure of sterilized skim milk, and (c) exposure of inoculated agar surface. Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for the air-borne phage although whey contaminated dust probably also played a part. Protection of the starter from air-borne phage eliminated phage failures. A separate building for starter propagation is suggested as the only means of insuring this at present. S.T.C.

685. The Consistency of Cheese Curd at the Pitching Point and Its Bearing on the Firmness and Quality of the Finished Cheese. G. W. Scott Blair and F. M. V. Coppen, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 44–54. 1941.

Further studies are reported on the use of the method previously described (Jour. Dairy Sci., *24*, No. 2: A26. 1941) for measuring the consistency of cheese curd at the "pitching" point. The value determined W/h was found to be an excellent criterion of the properties of the cheese curd assessed by an expert cheese maker.

No significant relationship was found to exist between the firmness of the curd at "pitching" and acidity. The most usual values (medians) for "pitching" consistency were compared for 4 different factories and it was shown that differences in technique may be associated with the same "pitching" consistency and produce very similar cheese, but that in other cases good cheese may be produced from very different "pitching" consistencies. S.T.C.

686. "Slowness" in Cheesemaking. J. Harrison and D. V. Dearden, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 35–43. 1941.

Two cases of "slowness" in cheesemaking are reported in which the trouble was eliminated by changing the source of the starter. The principal cause of the "slowness" studied appears to have been the inability of the streptococci in the starter cultures used to grow at scalding (cooking) temperatures. The necessity of using a starter capable of normal growth at 40° C. (104° F.) is thus demonstrated.

Attempts to isolate either bacteriophage or "non-acid" organisms failed. S.T.C.

687. The Problem of Rancidity in Cheddar Cheese. E. G. Hood, I. Hlynka and C. A. Gibson, Dept. of Agr., Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 3: 26. 1941.

The odor and flavor of rancid cheese are characteristic of those of butyric acid. It has been shown experimentally that it is possible to produce rancidity by the addition of butyric acid to the cheese milk. Lipase gives rise to butyric and other fatty acids when added to cheese milk. Raw milk contains lipase. Free butyric acid may be produced from casein, lactose, glycerol or butterfat. The most likely cause of rancidity is the action of lipase on butterfat to produce free butyric acid. In an experimental study of the problem it was found:

1. Typical rancid cheese were reproduced with the addition of lipase or homogenized milk to the cheese milk. Homogenization activates the naturally present lipase.
2. Rennet and pepsin partially inactivated additions to cheese milk of lipase or homogenized milk.
3. A number of the cheese made from inactivated milk fell in grade after a storage period of 6 weeks.
4. A relation between unclean, dirty, etc., flavors and rancidity in cheese was suggested. O.F.G.

688. Some Micro-organisms Associated with Gassy Swiss Cheese. Harry H. Weiser, Ohio State Univ., Columbus, Ohio. Natl. Butter and Cheese Jour., *32,* No. 7: 20. 1941.

Splitting rinds were observed on the edge and extending 4 to 5 inches toward the center of the Swiss cheese. The defect was accompanied by gas formation, lack of characteristic eye formation, poor body, bitter flavor, sometimes yeasty odor and lack of Swiss flavor. Bacteriological examination disclosed that associated with the occurrence of this defect were considerable numbers of lactose fermenting yeasts and anaerobic or facultative anaerobic bacteria of the *Clostridium perfringens* group. Other organisms may be involved. Practical control lies in the use of good milk and good active starter. W.V.P.

689. Pasteurization for Cheesemaking. G. S. Bixby, Cherry-Burrell Corp., Chicago, Ill. Natl. Butter and Cheese Jour., *32,* No. 7: 14. 1941.

Three types of pasteurizers commonly used are: the internal tube heater with tubular surface regenerator; a flash pasteurizer with a tubular surface type regenerator; and the plate pasteurizer. All are of the "flash" type and use maximum temperatures of 160°–165° F. frequently with a 16 to 20 second hold for increased bacterial destruction. Costs of pasteurizing

100 lbs. of milk approximate 1.5 cents for steam and 0.35 cents for power. Two to four man hours daily are required for cleaning. Extra costs are offset by benefits of increased yield, uniformity and quality. Health authorities, eventually, will demand that all cheese milk be pasteurized as Kentucky does now. W.V.P.

690. **Survey of Cheese Preferences.** EDITORIAL. Amer. Butter Rev., *3*, No. 5: 162, 196. May, 1941.

This editorial presents results secured in a survey representing 3.5 per cent of the families in Milwaukee, Wisconsin, as to their cheese preferences. Families purchasing packaged cheese in 1937 were 59.9 per cent and in 1940, 56.1 per cent, the numbers being 112,701 in 1937, and 110,569 in 1940. Of package cheese users, 48.8 per cent preferred the 8-ounce package, 26.5 per cent a smaller size, 12.4 per cent a 2-ounce carton, and 12.3 per cent preferred a one-pound package. In 1937, 82.6 per cent of the population surveyed regularly used bulk or loaf cheese; in 1940 the number had increased to 92.9 per cent. Of bulk cheese purchased 64.2 per cent was American type of cheddar, 22.9 per cent brick, and 12.9 per cent Swiss. Average consumption of bulk cheese per family has shown a variation of only one-tenth of a pound since 1934, monthly consumption per family being 2.1 pounds.

P.S.L.

691. **Manufacture of Acid-rennet Type Cottage Cheese.** D. W. GLOVER AND L. H. BURGWALD, Ohio State Univ., Columbus, Ohio. Milk Dealer, *30*, No. 8: 42–50. May, 1941.

Directions are given for the manufacture of acid-rennet type cottage cheese. Directions for using homogenized milk returns are included. The authors draw the following conclusions:

1. Start with sweet, clean skim milk and pasteurize it by heating to 143° F. for 30 minutes. Higher pasteurizing temperatures may be detrimental to the texture of the resulting curd.
2. Temper the milk to 70° F. if the long set is to be used or to 85° if the short set is to be employed.
3. Add starter at the rate of 0.5 per cent of the weight of the milk used for the long set or 3.0 per cent to 5.0 per cent for the short set. A choice between the long and short set methods will depend upon the work schedule of the particular plant.
4. Add rennet at the rate of 1.0 cc. per 1,000 pounds of milk in the vat.
5. Utmost care should be exercised in taking the whey sample for the acidity test to insure that no curd particles are present. The use of the whey well affords a satisfactory method of obtaining the sample.
6. Cut the curd when the acidity of the whey reaches 0.52 per cent.

7. The cooking may be accomplished by the use of hot water in the jacket of the vat and by adding hot water directly to the curd. The use of water in the cooking process enhances the firming of the curd, thus saving time in the cooking process.

8. The use of tempered wash waters prevents matting and the breaking up of curd made brittle by too sudden cooling.

9. The curd should be thoroughly chilled before creaming and packaging. Unchilled curd has a tendency to be tender and is prone to break up in the creaming process.

10. The use of homogenized milk results in a product with superior quality, and at the same time provides a method for utilizing returns.

C.J.B.

CHEMISTRY

692. Analysis of Proteins. 13. Caseo-Phosphopeptone. J. Lowndes, T. J. Rew Macara and R. H. A. Plimmer, St. Thomas's Hospital Medical School, London, S. E. Biochem. Jour., *35*, No. 3: 315–319. March, 1941.

Judging from its N and amino-N contents, caseo-phosphopeptone is an octapeptide containing two H_3PO_4 groups, 2 mol. glutamic acid and 2 mol. serine. Its acidity indicates the presence of another mol. of a dicarboxylic acid, leaving 3 mol. of simple amino-acids. V.C.S.

693. Methods of Measuring the Rate and Extent of Oxidation of Fats. Frank C. Vibrans, Amer. Meat Institute, 59 East Van Buren St., Chicago. Oil and Soap, *18*, No. 5: 109. May, 1941.

This paper discusses the following tests and methods for measuring the rate and extent of fat oxidation: 1. Kreis test; 2. Issoglio-Kerr test; 3. Aeration methods; 4. Photochemical methods; 5. Oxygen absorption; 6. Oxygen absorption-Peroxide method. V.C.S.

694. A Convenient and Efficient Method for the Determination of the Digestibility of Fats with Pancreatic or Other Lipases. J. R. Koch and Sister M. Dolorosa Duellman, Marquette Univ., Milwaukee, Wis. Oil and Soap, *18*, No. 4: 86. Apr., 1941.

A convenient and easy to operate method for carrying out hydrolysis experiments with pancreatic lipase is described. The authors list the following advantages for this method:

1. It allows five determinations to be carried out at one time.
2. It shows a smooth course of reaction in every case.
3. It gives more complete hydrolysis since the acids are used up as formed.

4. It keeps the pH on the alkaline side under constant control and in the range where the enzyme is most active.

5. It eliminates the removal of aliquots and the killing of the enzyme.

6. It makes it possible to make determinations directly in the digestion mixture. V.C.S.

695. **Antioxidants for Edible Fats and Oils.** H. S. OLCOTT, Mellon Institute, Pittsburgh, Pa. Oil and Soap, *18*, No. 4: 77. Apr., 1941.

Ascorbic acid, vitamin C, possesses antioxidant activity of the acid type while the tocopherols, that is vitamin E, possess the properties assigned to the phenolic inhibitors. Theoretically, combinations of the two should be particularly advantageous antioxidants, and actually the data confirm this assumption.

Purified lecithin possesses no antioxidant activity but with the commercial product the cephalin fraction carries the inhibitor action.

Cereal flours and particularly oat flour possess antioxidant properties. Phospholipids may account for part but not all of the effective principle of oat flour.

Cottonseed meal is an excellent antioxidant in fats and oils. V.C.S.

696. **The Mechanism of the Autoxidation of Fats.** H. A. MATTILL, State University of Iowa, Iowa City, Iowa. Oil and Soap, *18*, No. 4: 73. Apr., 1941.

Although the chemical changes during the induction period are very different and less obvious than those that follow it, they are, from a practical point of view, more important because once the induction period is past the damage is done. During oxidation the fats pass through two stages: a latent or induction period of variable length during which the amount of oxygen absorbed is small, followed by a period of rapidly accelerating oxygen absorption. The end of the induction period usually coincides with or immediately precedes the first appearance of the products of organoleptic rancidity.

Numerous tests have been devised for detecting the degree of susceptibility of a fat toward oxidation. These tests are based upon the estimation of some chemical change, yet the order of reason for the reaction is not clearly understood.

This paper deals with some of these chemical changes. No all-inclusive theory of autoxidation can yet be formulated. V.C.S.

697. **A Convenient Method for the Rapid Estimation of Carotene in Butterfat.** WILLIS D. GALLUP AND A. H. KUHLMAN, Oklahoma Agr. Expt. Sta., Stillwater, Okla. Oil and Soap, *18*, No. 4: 71. Apr., 1941.

A simple method is described for determining the carotene content of

butterfat under conditions where extreme accuracy is not required. A direct comparison is made of the color of the melted fat with that of known concentrations of potassium dichromate solution.

The dichromate solutions are prepared by dilution of measured amounts of a 0.2 per cent stock solution to a volume of 25 ml. These dilute solutions and the fat samples are contained in cylindrical sample bottles of uniform diameters and their color matched with the aid of a comparator block placed before a "daylight" lamp.

The authors give a table showing the carotene content of butterfat in micrograms per gram corresponding to the color produced by various concentrations of potassium dichromate. The carotene content may be calculated from the formula $X = \frac{Y - b}{a}$ in which X is the micrograms of carotene per gram of fat, Y is the concentration of the matching dichromate solution in per cent, and b and a are factors, 0.012 and 0.018, respectively.

V.C.S.

698. The Melting Points of Binary Mixtures of Oleic, Linoleic, and Linolenic Acids. H. W. STEWART AND D. H. WHEELER. Oil and Soap, *18*, No. 4: 69. Apr., 1941.

The oleic-linoleic acid system has eutectics for the alpha and beta forms of oleic acid of 75.2 and 76.3 mole per cent linoleic acid, at $-10.0°$ and $-9.8°$, respectively.

Linoleic and linolenic acid mixtures show only melting points intermediate between the pure acids.

The oleic-linolenic acid system has eutectics for the alpha and beta forms of oleic acid of 82.7 and 85.5 mole per cent linolenic acid, at $-15.7°$ and $15.1°$, respectively.

V.C.S.

699. Enzymic Proteolysis. 4. Amino-Acids of Casein Phosphopeptone. M. DAMODARAN AND B. V. RAMACHANDRAN, Univ. Biochem. Lab., Chepank, Madras. Biochem. Jour., *35*, Nos. 1 and 2: 122–133. Jan., 1941.

By digestion of "paranuclein" from casein with trypsin an enzyme-resistant phosphopeptone of constant composition had been isolated in the form of its barium salt.

The phosphopeptone was shown to contain 10 amino-acid units, viz., 3 mol. glutamic acid, 3 mol. of isoleucine and 4 mol. of serine. The absence of other hydroxy- or dicarboxylic-amino-acids has been demonstrated by indirect methods.

A method is described for the approximate estimation of serine in the absence of other hydroxyamino acids.

V.C.S.

700. **The Component Acids of Phosphatides Present in Cow's Milk Fat.** THOMAS PERCY HILDITCH AND LIONEL MADDISON, Dept. Indust. Chem., Univ. Liverpool. Biochem. Jour., *35,* Nos. 1 and 2: 24–30. Jan., 1941.

The typical milk fat glyceride acids of low molecular weight are wholly absent from the phosphatide acids. The component fatty acids of milk fat phosphatides have little in common with those of the milk fat glycerides; on the other hand, they bear more general similarity to those of the phosphatides of the ox liver.

The authors report the following acids and amounts in the phosphatides separated from Swiss and English butters:

Acid	Per cent by weight	
	"Swiss"	"English"
Myristic	3.2	5.5
Palmitic	21.0	13.4
Stearic	7.3	9.0
As Arachidic	12.3	20.9
As $C_{26}H_{52}O_2$	5.2	10.0
Hexadecenoic	4.3	4.9
Oleic	32.5	23.5
As Octadecadienoic	6.4	......
As C_{20-22} unsaturated	7.8	12.8

V.C.S.

701. **Preliminary Experiments on the Vapor Pressure of Dairy Products.** G. W. SCOTT BLAIR, F. J. DIX AND A. WAGSTAFF, Natl. Inst. Res., in Dairying, Univ. Reading, Reading. Eng. Jour. Dairy Res., *12,* No. 1: 55–62. 1941.

The vapor pressure was determined as follows: A large number of airtight tobacco tins of diameter about 4.5 in. were fitted with a simple wire clip to hold a pad of cotton wool about 2 in. square, against the lower side of the lid. The cheese were spread thinly on the bottom of the weighed tin, which was then reweighed as quickly as possible to avoid evaporation. A pad of cotton wool was then soaked in a salt solution of known concentration and vapor pressure and was lightly pressed out to prevent dripping. The pad was quickly clipped against the lid and the tin tightly closed. After 48 hours at a constant temperature, approximately 60° F. (15.6° C.), the lid was removed and each tin and cheese reweighed immediately. The change in weight per gram of cheese was then plotted against salt concentration and that concentration corresponding to no change in weight was obtained by interpolation.

Vapor pressure moisture curves are given for a number of different varieties of cheese. The vapor curve was found to be influenced by the

amount of salt in the cheese, but differences between varieties cannot be accounted for entirely in terms of differences in salt content.

A preliminary experiment on the relationship between vapor pressure of Stilton cheese and amount of blueing indicated that such a relationship does in fact exist, but that a much larger experiment is required before the connection is fully understood.

Preliminary experiments on the measurement of the vapor pressure of milk showed that additions of 2–3 per cent of water in milk can be detected.

S.T.C.

702. **Electrical Testing Device.** Nathan Schnoll. Amer. Milk Rev., *3*, No. 5: 106, 107. May, 1941.

As a method for checking the percentage of free caustic in a washing machine the solubridge is a modified Wheatstone bridge for measuring conductivity of caustic solutions. By using a rotary switch different tanks of caustic solutions may be checked with the same bridge. Used with a total alkalinity test it gives positive knowledge of the strength of the washing solution. P.S.L.

703. **Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus.** W. R. Johnson and Charles N. Frey, Fleischmann Labs., Standard Brands, Inc., New York, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., *13*, No. 7: 479–481. 1941.

The Barcroft-Warburg equipment was used to measure induction periods of sesame and cottonseed oils at temperatures from 50° C. to 100° C., most of the determinations being made at 100° C. in an atmosphere of oxygen. The data show that the Barcroft-Warburg technique can be extended to elevated temperatures with convenience and precision. B.H.W.

704. **Determination of Thiamin by the Thiochrome Reaction.** R. T. Connor and G. J. Straub, Central Labs., General Foods Corp., Hoboken, N. J. Jour. Indus. and Engin. Chem., Analyt., Ed., *13*, No. 6: 380–384. 1941.

This method for the determination of thiamin first proposed by Jansen is based on the measurement of the florescence produced by thiochrome formed by the oxidation of thiamin with potassium ferricyanide in an alkaline solution. This paper defines more exactly than has been done previously, the optimal conditions for carrying out the thiochrome procedure and suggests some improvement in the equipment used. Extraction and hydrolysis of the sample are carried out in the same vessel and for the enzymatic hydrolysis of cocarboxylase, the enzyme clarase is introduced. Optimal conditions for the oxidation of thiamin and for the extraction of the thio-

chrome formed are given. The method is in close agreement with biological assays and has been applied to various types of natural products including whey and skim milk powders. B.H.W.

705. **Combined Determination of Riboflavin and Thiamin in Food Products.** R. T. CONNOR AND G. J. STRAUB, Central Labs., General Foods Corp., Hoboken, N. J. Indus. and Engin. Chem., Analyt. Ed., *13,* No. 6: 385–388. 1941.

A rapid and accurate procedure is described which makes possible the determination of both vitamins on the same sample. The method is an extension of the one proposed for thiamin and gives results closely agreeing with biological assays. It has been applied to grains, dairy products, fresh and frozen vegetables. Rapid destruction of riboflavin in aqueous solutions by light was found. Destruction by diffused light of the laboratory occurred irrespective of pH but in artificial light destruction was slower and dependent upon pH. The pH range from 2 to 8 was studied. Ferrebee's procedure for adsorption of riboflavin on Supersorb was modified to use a smaller extraction column and a study was made of Corning glass filters suitable for the fluorometric determination of riboflavin. B.H.W.

706. **Distribution of Nitrogen and Protein Amino Acids in Human and Cow's Milk.** ELIOT F. BEACH, SAMUEL S. BERNSTEIN, OLIVE D. HOFFMAN, D. MAXWELL TEAGUE AND ICIE, G. MACY, Res. Lab. Children's Fund of Michigan, Detroit. Jour. Biol. Chem., *139,* No. 1: 57. May, 1941.

The amounts (in milligrams) of seven amino acids contained in the proteins of 100 ml. of human and cow's milk were calculated to be as follows:

	Cow's milk	Human milk
Histidine	59	12
Arginine	127	40
Lysine	223	50
Tyrosine	197	50
Tryptophane	43	19
Cystine	23	20
Methionine	104	18

In the proteins of cow's milk the preponderance of sulphur is in the form of methionine, with very little in the form of cystine, while in the proteins of human milk the sulphur is about equally divided between cystine and methionine. V.C.S.

DISEASE

707. **Lancefield Group B Streptococci (Str. agalactiae) on the Hands of Milkers and Others.** J. HARRISON, Natl. Inst. Res. in Dairying,

Univ. Reading, Reading, Eng. Jour. Dairy Res., *12,* No. 1: 18–23. 1941.

Lancefield group B streptococci were recovered from the hands of all but one of the milkers (eight in all) on three different farms. A routine method of disinfecting the milkers' hands consisting of a soap and water wash followed by a rinse in sodium hypochlorite solution containing about 800 parts per million available chlorine was used on the farms. Following, in most instances, the routine cleaning, the hands were scrubbed with a hard nail brush in sterile milk and the milk examined for the group B streptococci using Edwards medium.

Since the organisms recovered from the hands of the milkers had the biochemical reaction of *Str. agalactiae*, and since none was recovered from the hands of non-milkers, the milkers' hands were considered to be a potential source of infection to cows. S.T.C.

708. Die Agglutinationsmethode nach Stableforth und Willems zur Feststellung der Rinderbruzellose. (The Agglutination Test of Stableforth and Willems in the Diagnosis of Brucellosis in Cattle. R. ENDRESS, (Aus der vet.-med. Abteilung d. Reichsgesundheitsamtes, Zweigstätte Dahlem.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *56,* No. 4: 297–320. 1940.

Since the agglutination test is the most important and widely used test for the diagnosis of this disease, attempts have been made to standardize it for international use. A standard test is necessary because of the great variation in methods used by different workers. Stableforth in London, and Willems in Brussels, have suggested standard methods for making the tests and this investigation was an attempt to evaluate them.

This writer used five strains of *B. bovis.* The cultures were grown on 2 per cent glycerine agar, pH 7.5, for 3–4 days at 37° C. The suspension was made in phenolized salt solution, filtered through gauze, and heated at 70° C. to kill the organisms. It was examined culturally and microscopically for purity. This suspension was standardized by a Leitz photometer to the density recommended by Stableforth (Brown No. 4 = 97 cc. 1 per cent H_2SO_4 plus 3 cc. 1 per cent $BaCl_2$ solution). The suspension was placed in the refrigerator 2–3 weeks at 5–6° C. and the agglutinability tested with a known serum. Stableforth kept a dried serum to which was added sterile saline at time of use to give dilutions of 1:25 to 1:750 in 30 tubes. To the various serum dilutions was added an equal volume of the antigen, thus doubling the dilution. Appropriate controls were included. The tests were incubated at 37° C. for 24 hours and for one hour at room temperature.

As a guide in the evalution of the antigen, Willems' modification of the formula of Stableforth was used. This consists of determining the agglu-

tination constant and the agglutinability index. In the agglutinability index the titer limit of a suspension of the antigen and a standard serum is observed. The agglutination constant is the relation between the diagnostic titer of the infection and the agglutinability index. According to Willems the former is 50 and the agglutinability index for his particular antigen was 700; therefore, the agglutination constant was $50/700 = 0.07142$. With the aid of a standard serum the agglutinability index of other antigens can be determined. By multiplying the numbers thus obtained by the agglutination constant the infection titer can be determined. The agglutinability index for the English strains of the organism studied was 500, for the German strains 550. The infection titer for the latter strain was $550 \times 0.07142 = 39.3 = 40$. A 25 per cent agglutination in a 1:40 dilution was considered as a positive reaction.

The sensitivity of all cultures used in preparing the antigens was tested before they were used. In routine testing, dilutions of the serum are made by using 1 cc. of serum and 4 cc. of 0.5 per cent salt solution. This is diluted to 1:80 or above. In this investigation 1:40 was considered as the infection titer.

From this study it was concluded that the agglutination method of Stableforth and Willems for the diagnosis of brucellosis in cattle is useful and reliable.

The advantage of the method lies in the accuracy and greater ease of determining the results. For the diagnosis in doubtful cases the method has an advantage.

The utilization of several strains of bacteria in preparing the antigen was considered necessary in order to make it as active as possible.

The difference obtained by the use of the Stableforth-Willems method and those methods now commonly used in Germany and the complement fixation test are negligible. L.D.B.

709. **The Role of Milk in Tuberculosis.** H. A. Reisman, Queens General Hospital, Jamaica, N. Y. Certified Milk, *16*, No. 179: 5. March, 1941.

Milk not only plays an important role as the vehicle through which this disease is transmitted to man, but it also plays an important role in the treatment of infected individuals, because of the availability of its calcium. The author concludes that the bovine strains of tuberculosis in man can be completely eliminated in two ways:

(1) The eradication of the disease at its origin by tuberculin testing, and the slaughtering of all positive reactors.

(2) By the universal pasteurization of all market milk. Since there is evidence to show that pasteurization does alter the milk, it would seem that the interest of health should demand a good, fresh, clean milk at the start.

W.S.M.

710. **What an Inspector Should Look for in Making Dairy Cattle Physical Examination.** C. U. DUCKWORTH, State Dept. Agr., Sacramento, Calif. Jour. Milk Technol., *4*, No. 1: 48. 1941.

An inspector should form the habit upon entering the stable to observe each animal carefully. The physical examination should take in manifest evidence of disease, such as enlarged glands, mastitis, pyometra, tuberculosis, ulcerated teeth, abscesses, and any abnormality in general.

Veterinary council is to be advised where any question occurs with respect to specific nature of any condition found. L.H.B.

711. **Experimentelle Bruzellose beim Hunde. (Experimental Brucellosis in the Dog.)** WALTER DOMKE (Aus dem Hygienischen institut der Tierärztlichen Hodhscule Hannover.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *56*, No. 4: 321–328. 1940.

The views concerning the abortus bacillus infection in the dog and the possibility of this animal being a disseminator of the organism are conflicting. The serological evidence on this point is very limited. Some writers on the subject consider that there may be certain factors which will influence the sensitivity of these animals to this infection.

Dogs of different ages were fed and inoculated with the organism. Clinical observations and agglutination tests were made for three-month periods when the animals were killed and cultural and pathological studies made. In order to test the localizations of the organisms suspensions of the sex organs were made in salt solution and 2 cc. injected into guinea pigs. These animals were then examined for 6 weeks when they were killed and cultural and serological examinations made.

From this investigation it was concluded that the dog is susceptible to *Brucella bovis* infections by feeding and injection. Clinically the course of the disease in the dog is much the same as in man. Pathological changes were observed in the genital organs. The agglutination titer followed very closely the fever curve. Gravid females were more susceptible than others.

Pathologically the disease in dogs is similar to that in cattle in that the organisms localized in the placental membranes.

An agglutination titer of 1: 80 was observed in some of the infected animals. L.D.B.

712. **Erfahrungsbericht über die amtlichen tierärztlichen Milchuntersuchungen aus dem Jahre 1937–1938 in Regierungsbezirk Oberschlesien. (Report of the Official Veterinary Milk Investigations for 1937–1938 in Upper Silesia.)** OTTO SCHIEL (Aus dem Staalichen Veterinär-Untersuchungsamt Oppeln). Ztschr. f. In-

fektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *57*, No. 2: 159–176. 1941.

This issue contains only the first part of the paper; the final part will appear in the next issue of this journal.

The report is detailed and contains considerable information on the results of official investigations of the milk supply of the area together with a scheme for control of milk-borne diseases. The report deals with the finding of tubercle bacilli, the Bang's disease organism and mastitis streptococci.

It has not been possible to control tuberculosis entirely in these larger areas and the official veterinary control service has developed plans for a more detailed study of the disease producing organisms and methods of control in a limited area. The study covered the years 1937 and 1938. Udder tuberculosis was found in 1.92 per cent of 3,381 samples from 61 different sources. Abortion bacilli were found in 8.21 per cent of these samples. Microscopic examination revealed 1.16 per cent contained mastitis streptococci. There was considerable variation in the results obtained with milk from different sources.

Conclusions will appear in the next issue. L.D.B.

713. **Zur Feststellung von Tuberkelbazillen in Ausscheidungsproben vom Rind. (A Study to Determine the Presence of Tubercle Bacilli in the Excreta of Cattle.)** W. Stockmayer (Aus dem Württ. Tierärztlichen Landesuntersuchungsamt.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *57*, No. 1: 75–84. 1940.

The older methods of detecting the presence of tubercle bacilli in excreta by animal inoculation is tedious, time-consuming and costly. The writer has developed other methods which he considers as satisfactory substitutes. The methods here described are microscopic, cultural and animal inoculation.

The microscopic methods may be direct, using fresh material or following an enrichment. A combination of the two appears preferable to either alone. The writer examined 202 samples of bronchial slime from tuberculous animals by means of the antiformin method using 2.5 per cent concentration of the chemical. The material was obtained with a tracheal cannula and swab. The sample thus obtained by means of a swab was spread directly on slides. The swab was then placed in 2.5 per cent antiformin for 20 minutes and squeezed out with sterile forceps. The fluid thus obtained was centrifuged and the sediment stained by the Ziehl-Neelsen·method and examined. Of the 202 samples studied, 119 were positive by direct examination and 113 by the so-called enrichment process. In 41 instances the direct examination was most strongly positive and in 24 instances the

enrichment method gave better results. The reason the direct examinations gave more positive results was that the organisms were in clumps and more easily found. Also, the antiformin treatment tends to reduce the intensity of the staining.

The usual method of injecting animals and holding them for eight weeks with frequent examinations and removal of regional lymphatics is time-consuming. An attempt was made to reduce this period. The material obtained on a swab was placed in 1 cc. salt solution and centrifuged and the sediment suspended in 1 cc. of the cream obtained from 60 cc. of milk. This material was injected into guinea pigs and the animals examined at weekly intervals. When enlarged lymph nodes could be observed they were recovered under local anaesthesia and examined microscopically for the presence of tubercle bacilli. All negative glands were examined histologically and the presence of giant cells was considered as positive. The writer concludes that holding the animals for six weeks is not sufficient.

For cultures the writer used sediments obtained by suspending the slime in an HCl solution (15 cc. HCl sp. gr. 1.122 plus 83 cc. aq. dist.) from five to 15 minutes with shaking followed by centrifugation for 10 minutes. The sediment was then placed on Patragnani-Witte media. It was found that this concentration of HCl did not destroy the tubercle bacilli in the time used. There was good agreement between the results of culture and animal inoculation. The culture usually gave 20–30 per cent more positive results than the microscopic examination.

As a result of this investigation the writer concludes that animal inoculation gives the highest results and that the guinea pigs used in such investigations should be held for eight weeks or longer. L.D.B.

FOOD VALUE OF DAIRY PRODUCTS

714. Effect of Milk on Gizzard Erosion and Cholic Acid in the Chick. H. J. Almquist, E. Mecchi and F. H. Kratzer, College of Agr., Univ. California, Berkeley. Soc. Expt. Biol. and Med. Proc., *47:* 525. 1941.

Evidence was presented for the existence in cows milk of a labile substance which acts like cholic acid. Dried milk products were fed by mixing in the diets while liquid milk products were given to the chicks in place of the drinking water. Liquid milk products reduced the severity of gizzard erosion, increased the gall bladder bile volume per chick and increased the quantity of cholic acid per chick. There was no effect on the characteristics measured following the feeding of dried milk products. Attempts to detect cholic acid in skim milk produced only negative results.

R.P.R.

715. **The Effect of Vitamin A Intake on Vitamin A Content of Butterfat.** HARRY J. DEUEL, JR., NELLIE HALLIDAY, LOIS HALLMAN, CORNELIA JOHNSTON AND ALBERT J. MILLER, Dept. of Biochemistry, Univ. of Southern California, Los Angeles, Calif. Jour. Biol. Chem., *139*, No. 1: 479. May, 1941.

The supplementary feeding of vitamin A in the form of Shark liver oil greatly increased the vitamin A content in the butterfat. The milk production of the cows receiving the vitamin A supplement promptly rose and continued at a level approximately 10 per cent higher than the cows not receiving the vitamin A during the test. V.C.S.

716. **Proteins and Our Dairy Products.** F. H. PLETCHER, Lab., Borden Farm Products, Brooklyn, N. Y. Milk Dealer, *30*, No. 8: 36, 56–60. May, 1941.

Proteins are discussed under the following headings: Why Stress Proteins? What is Protoplasm? Chemical Composition. Amino Acids. Milk Proteins. Needs of Adults. The author summarizes his discussion as follows:

Why stress the importance of proteins in our dairy products when there are also present the all important minerals, and excellent carbohydrate, butterfat and vitamins in varying quantities? Possibly because we seldom realize we handle this food nutriment—when we think of milk our cothought is usually fat and sometimes total solids.

Proteins, however, are highly important in the diet—especially the milk proteins, for in milk we have the "complete" proteins, capable of maintaining life and supporting growth. Other foods are rich in protein, but they are usually lacking in some essential amino acid and are therefore either "incomplete" or "partially incomplete." An example of a food rich in "incomplete" proteins is gelatine, which will neither maintain life nor support growth; cereals, on the other hand, are generally classed as "partially incomplete" since they are usually lacking in at least one essential amino acid which is necessary for the promotion of growth, although they will maintain life.

But in milk or other dairy products such as cottage cheese we have protein foods containing all of the known 23 amino acids which when included in the dietary, in sufficient amounts, have been proved by experimentation to possess outstanding properties.

It's up to the men in the dairy industry to educate consumers on this point. C.J.B.

717. **Effect of Supplemented Raw and Pasteurized Milks upon Growth and Well-being of Rats.** ALICE M. BAHRS, St. Helen's Hall Junior

College, Portland, Ore., AND ROSALIND WULZEN, Orgeon State College, Corvallis, Ore. Certified Milk, *16,* No. 180: 5. Apr., 1941.

Rats fed raw milk rations showed a superior growth and certain tissues of the body showed distinct differences. W.S.M.

718. **The Soil Basis of Better Milk Production.** L. A. MAYNARD, Cornell Univ., Ithaca, N. Y. Certified Milk, *16,* No. 181: 5. May, 1941.

The author discusses ways in which the soil is definitely related to milk quality. It is generally understood that the nature of the feed of the cow affects the quality of the milk as well as its quantity. But, it is less appreciated that the quality factor in the feed depends upon how the feed crop is produced and particularly upon the fertility of the soil. The influence of the soil on milk quality is an indirect one, expressing itself through the food crop, yet it has some advantages over adding deficient nutritive essentials directly to the feed. W.S.M.

719. **Possibilities of Improving Milk by Increased Nutritive Qualities in Feeds.** D. B. HAND, Cornell Univ., Ithaca, N. Y. Certified Milk, *16,* No. 182: 7. June, 1941.

The author concludes that iodine and a few of the fat soluble constituents, notably vitamins A and D, are the only substances in milk which are greatly dependent on feed. From the practical standpoint for some of the other constituents, it is possible to improve the quality of milk by selection of individual cows. However, selection of cows offers many complicated problems. The quality of milk with respect to color and flavor can be improved by feeding. How many compounds are present in milk and which of these are essential to human diet is still unknown. W.S.M.

720. **Nutritional Restoration and Fortification of Foods.** Jour. Indus. and Engin. Chem., *33,* No. 6: 707–722. 1941.

Several papers on this subject which are of general interest were presented in a symposium at the 101st meeting of the American Chemical Society, St. Louis, Missouri. These papers are as follows:

Nutritional Requirements of Man. C. A. Elvehjem, Univ. of Wisconsin, Madison, Wis.

Cereal Products. R. T. Connor, General Foods Corp., Hoboken, N. J.

Fortification and Restoration in the Baking and Dairy Industries. James A. Tobey and William H. Cathcart, Amer. Inst. of Baking, New York and Chicago.

What the Consumer Should Know about Fortified Foods. Helen S. Mitchell, Nutrition Div., Health, Welfare and Activities Affecting National Defense, Washington, D. C.

Fortification and Restoration of Processed Foods. R. R. Williams, Bell Telephone Labs, New York, N. Y.

Control Problems of the National Nutrition Program. E. M. Nelson, U. S. Food and Drug Admin., Washington, D. C. B.H.W.

ICE CREAM

721. **Helping the Ice Cream Retailer Stay in Business.** John Kirkwood, Advertising and Marketing Counsellor, Toronto, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 52. 1941.

The author discusses rather lengthily the principles of a retail business. Although he points out that 75 per cent of retailers do not last 7 years, he states that retailing can be a "safe" business if business principles are charted and the chart used as a guide. O.F.G.

722. **Some Suggestions for Stepping up Winter Sales of Ice Cream.** Anonymous. Ice Cream Rev., *24,* No. 7: 94. 1941.

Suggestions are given for increasing the use of ice cream during the winter months by means of health appeal, colored mailing pieces and magazine advertisements. J.H.E.

723. **Ice Cream Production by Regions for the Years 1930–1939.** E. E. Vial, Bureau of Agr. Econ., U. S. D. A., Washington, D. C. Ice Cream Rev., *24,* No. 7: 84. 1941.

Data, gathered by the Agricultural Marketing Service, is compiled showing ice cream production and per capita consumption for principal geographic areas. These data indicate that per capita consumption of commercial ice cream is highest in the North Atlantic states, amounting to 3.02 gallons in 1939. The South Central states were lowest with 1.17 gallons per capita in the same year. J.H.E.

724. **Sugars That Can Be Used in Ice Cream Making.** P. H. Tracy, Univ. Illinois, Urbana, Ill. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 68. 1941.

Sugars, which compose approximately 20 per cent of the weight of ice cream, perform several rather important functions when used in ice cream. They are high in energy value and greatly increase the palatability of the ice cream. They sufficiently lower the freezing point of the mix to permit incorporation of the desired amount of air without the semifrozen mass becoming too stiff to be removed from the freezer. Granulated cane and beet sugars are the most important sources of sweetness for ice cream. Generally the dry sugar has been used but in recent years a sugar syrup which contains about 68 per cent solids has found some favor. The advantages of the syrup are that it can be transported in tank trucks and

handled by pumps. Corn sugar, known as dextrose, glucose or cerelose, is a monosaccharide and differs in some of its properties as compared to cane or beet sugar, known as sucrose, which is a disaccharide. Dextrose is manufactured by hydrolyzing corn starch. It has a sweetening value of approximately 70 as compared to 100 for sucrose but its sweetening power is increased when used in conjunction with sucrose in ice cream. A new type of corn syrup, known as "Sweetose," recently has been perfected which has a much higher dextrose equivalent and better flavor than the older syrup. "Sweetose" has a beneficial effect upon the body of ice cream, sherbets and ices. A dry corn sweetening agent, known as "Fro-dex," has recently been introduced. In the selection of a sweetening agent the manufacturer of ice cream should be governed by cost as well as by the effect of the product upon the ice cream. O.F.G.

725. **Some Pointers in Making Sherbets and Water Ices.** P. H. TRACY, Univ. Illinois, Urbana, Ill. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 20. 1941.

Desirable features of a good stabilizer for ices and sherbets are given as follows: (1) Stabilizing qualities should not be impaired by citric acid, (2) should be easily dispersed, (3) should be a desirable effect upon texture and resistance, (4) should have sufficient effect upon viscosity to prevent settling out of unfrozen syrup, (5) should not cause high overrun, (6) should be tasteless.

The merits and methods of using such stabilizers as gelatin, gums, pectin and carob bean products are discussed. The amount of a monosaccharide sugar, such as honey, dextrose or sweetose, which can be used in conjunction with sucrose is limited to about 7 per cent. The following defects are discussed: bleeding, surface crustation, crumbly body, hard body, snowy body, coarse body and sticky body. O.F.G.

726. **Ice Cream Sales Index for 1941.** Statistical and Accounting Bureau. Internatl. Assoc. Ice Cream Mfrs., Washington, D. C. July, 1941.

This bulletin contains an analysis of ice cream sales in the United States and Canada for the first four months of 1941. The increase in sales over 1940 for the first four months of 1941 is as follows:

Month	Per cent of increase	
	United States	Canada
January	31.42	49.07
February	9.57	39.07
March	15.14	45.08
April	27.66	78.44
Average increase	21.05	56.52

The Central Eastern States led the increase for the four-month period with a gain of 28.4 per cent, the North Atlantic States, followed with 21.67 per cent increase over the previous year, the Midwestern States enjoyed a gain of 17.59 per cent, the Southern States 15.77 per cent, while in the Western States the increase was 4.76 per cent.

The bulletin also contains an analysis of business and weather conditions in the different sections of the country. In the supplement to the bulletin the final ice cream statistics for 1939 of the Agricultural Marketing Service of the U.S.D.A. are given. The total production of ice cream for 1939 was 304,522,000 gallons. M.J.M.

727. **The Frozen Desserts Code Recommended by the Public Health Service.** A. W. FUCHS, Sanitation Section, U. S. P. H. Service, Washington, D. C. Jour. Milk Technol., *4*, No. 1: 26. 1941.

The need for public health control of frozen desserts is cited. Prior to 1928 there have been 36 outbreaks of milk borne disease reported in the literature as having been traced to ice cream. In the five-year period, 1934 to 1938 inclusive, ten outbreaks have been reported.

The Public Health Service urges states not already doing so to adopt a frozen desserts control program similar to the milk control program. L.H.B.

728. **Chocolate Malted Milk.** C. E. HENDERSON, Bastian-Blessing Co. Ice Cream Rev., *24*, No. 6: 31. 1941.

The popularity of chocolate malted milk drinks is due to their delicious taste, high food value and the quick energy they provide. The variable factors are the chocolate flavor and the consistency of the finished drink. The consistency depends not only upon the proportions of milk and ice cream used, but also upon the temperature of the ingredients and the mixing time. Blending is done by whipping air into the milk as the ingredients are mixed. Milk at 32° F. can hold approximately 90 per cent air. Electric mixers accomplish mixing and aeration in less than two minutes. If the mixing is too rapid, the ice cream will be broken down instead of blended. If not removed from the mixer when the maximum aeration of the milk has been accomplished, the air will escape as the temperature of the milk rises. Success consists largely in keeping the ingredients as near the freezing temperature as possible during mixing.

A number of formulas are given. J.H.E.

729. **New Developments in the Science of Ice Cream Making.** W. H. MARTIN, Kansas State College, Manhattan, Kan. Ice Cream Rev., *24*, No. 6: 34. 1941.

This is a review of the recent problems and developments in ice cream

manufacture. Shrinkage, ingredients, melting qualities, stabilizers, flavors and sanitary quality are some of the subjects discussed. J.H.E.

730. **Sugars That May be Used in the Ice Cream Industry.** P. H. TRACY, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., *24*, No. 5: 29. 1940.

When the ice cream manufacturer replaces sucrose with other sweetening agents he should expect some differences in results. The type of sweetness may not be the same, but this is not necessarily a disadvantage. Some of the present replacement agents contain prosugars of high molecular weight so that the lowering of the freezing point of the mix is not a serious problem. It is claimed that in some cases a better flavor and a better body may result from the use of a sweetening agent made from corn. J.H.E.

731. **The Control of Shrinkage in Ice Cream.** J. H. ERB, Ohio State University, Columbus, Ohio. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 60. 1941.

The mechanism of ice cream shrinkage is fundamentally a problem of a destabilized protein or a protein sensitive to coagulation. Factors which operate to influence protein stability are, (1) salt balance of the original milk, (2) added salts, (3) acidity, (4) composition of the mix, (5) homogenizing pressures, (6) stiffness of freezing, and (7) hardening temperature and fluctuations in storage temperature. Another factor which influences shrinkage is the ease of air transfer. Mechanical factors which influence air transfer are (1) type of container, (2) jolting of truck, (3) amount of overrun, (4) size of air cells, and (5) external pressures. Factors making for a stable, or highly hydrated plastic protein are desirable in correcting shrinkage since such a protein better holds the air within the cells. O.F.G.

732. **Is There a Place for Substandard Products in the Ice Cream Industry?** C. H. SNOW, Snow and Palmer Co., Bloomington, Ill. Ice Cream Rev., *24*, No. 5: 74. 1940.

There are dairy products which are meritorious for which standards are desirable. Among these are "cereal cream" and a frozen malted milk mixture. Standards are created by custom of consumers and of the trade generally or they may be created by law.

The test of value in any specific product would seem to be whether or not its production and sale resulted in benefit to the producers of milk and the consumers of the product. Substandard or inferior commodities should not be substituted for the genuine, but there are places for additional standards for special products that fill a real need which is beneficial to industry.

J.H.E.

733. **Use of Vanilla and Other Flavors in Ice Cream.** ANONYMOUS. Ice Cream Rev., *24,* No. 7: 114. 1941.

This is a review of a paper written by Dr. A. Katz of Florasynth Laboratories, Los Angeles, California. In the inception of the ice cream industry, the ice cream was flavored with chopped vanilla beans, as the art of making extract was not known. In the early days of making vanilla extract 95 per cent alcohol was used. This destroyed the fine vegetable aromatic principles present in the vanilla beans. Best results are now obtained using 30 to 35 per cent alcohol.

To obtain proper results in citrus flavorings it is necessary to utilize not only the juice of the fruit but also flavor obtained from the peel. These should be combined together in a vegetable gum media.

Interesting facts about other flavors for ice cream such as butterscotch, English toffee, grenadine, etc., are included. J.H.E.

734. **Dextrose and Corn Syrup for Frozen Desserts.** A. C. DAHLBERG AND E. S. PENCZEK, New York Agr. Expt. Sta., Geneva, N. Y. Ice Cream Rev., *24,* No. 7: 38. 1941. (This is a review of the original New York Agr. Expt. Sta. Bul. No. 696, "Dextrose and Corn Syrup for Frozen Desserts" by the authors of this article.)

Good results can be secured when 25 per cent of the sucrose in ice cream is replaced with dextrose or corn syrup to give comparable sweetness. Based upon securing comparable sweetness the weight of the dry corn sweeteners required to replace one pound of sucrose is as follows: Enzyme-converted corn syrup, 1.5 pounds; corn syrup solids, 2.0 pounds, and dextrose, 1.1 pounds. J.H.E.

735. **Understanding Improves Consumer Friendship.** RACHAEL REED, The Borden Co., Chicago, Ill. Ice Cream Rev., *24,* No. 7: 34. 1941.

It is possible for the ice cream industry to profit by the experience of fluid milk and other industries in giving the consumers the information they would like to have about the ice cream industry and its products before they become too critical. A number of things consumers are interested in about ice cream are discussed. The article contains several good tables on the comparative food value of ice cream and other desserts. J.H.E.

736. **High Operating Costs.** C. F. BAKER, Atlanta, Ga. Ice Cream Rev., *24,* No. 7: 29. 1941.

Some of the common causes for high operation costs of the refrigeration system are malpractices in connection with pumping condenser water, low ammonia charge, high condensing pressures and oil in evaporating coils. Examples of these conditions and their remedy are given. J.H.E.

737. **Cherries, the Luxury Fruit.** HOWARD BLACK, Traverse City, Mich. Ice Cream Rev., *24*, No. 7: 26. 1941.

The author sketches some interesting history of cherries. Cherries are a valuable ice cream flavor because of the eye appeal. J.H.E.

738. **Formulas for Combination Flavors of Pineapple and Other Flavors.** ANONYMOUS. Ice Cream Rev. *24*, No. 6, 52. 1941.

A number of new ice cream flavors combining pineapple and other popular flavors has been developed by Dr. C. D. Dahle, of Pennsylvania State College. Detailed formulas and other helpful instructions are given for a number of combinations. J.H.E.

739. **Pasteurizing the Ice Cream Mix.** J. M. BRANNON, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., *24*, No. 6: 28. 1941.

The ice cream industry is endeavoring to fix a standard temperature and time for pasteurization of ice cream mix. It has been shown that a temperature of 150° F. for three minutes will kill *Eberthella typhi,* beta hemolytic streptococci, *Corynebacterium diphtheria* and bovine tubercular bacilli in ice cream mix. Twelve states have set bacterial standards for ice cream.

The results of a survey of Illinois ice cream made a few years ago by the author are cited which indicated only 17 per cent of the samples had bacterial counts of 100,000 or less. Sixty per cent of these samples gave a positive test for the coli aerogenes group. The author concludes that in the average plant the ice cream mix is generally sufficiently pasteurized but that large numbers of organisms are picked up from equipment after pasteurization. J.H.E.

740. **Cutting Truck Refrigeration Costs.** ANONYMOUS. Ice Cream Rev., *24*, No. 6: 26. 1941.

The experience of a southern ice cream company with a new group of five refrigerated trucks is discussed. Three of the five trucks are used for city delivery and are quipped with ammonia refrigeration coils. These are hooked up each day to take-off lines at the plant. Trucks on country routes, which do not return to the main plant every day, are equipped with compressors as well as power take-off units on the drive shaft. Savings have been especially realized for the country trucks because they do not lose efficiency when kept in the territory overlong. They can spend more time in actual service than formerly. J.H.E.

MILK

741. **Milk in the Schools.** F. W. HAMILTON, Royal Oak Dairy, Ltd., Hamilton, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 17. 1941.

The primary objective in the sale of milk in schools is not the sale of milk

but the development of the milk drinking habit. The school is the best place to develop in young people the habit of drinking milk instead of soft drinks, tea and coffee. A plan was worked out through the Board of Education whereby the dairies agreed to furnish refrigeration in the schools and in return were to be allowed to sell "free" milk to school children. The schools were apportioned to those competing dairies which wished to enter the plan. For the year 1938–1939, before the plan was put into effect, the amount of free milk given out was 273,000 half pints. For the year 1939–1940, after the plan went into effect, the free milk totaled 378,000 half pints and the sale milk totaled 285,000 half pints. O.F.G.

742. **Cooked Flavor in Milk, a Study of its Cause and Prevention.** I. A. Gould, Michigan State College, East Lansing. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., *21:* 553–564. Apr., 1941.

That the "sulfurous-like" flavor produced in milk by high temperature heat treatment is related to sulfur compounds is shown by producing the defect by adding to milk a sulfite salt or glutathione which contains the sulfhydryl (–SH) linkage and artificially producing a cooked flavor. Two methods were employed to determine if sulfur were involved (a) liberation of sulfides from the milk, (b) the nitro prusside test which detects the presence of sulfhydryl (–SH) groups for which a slight modification of the technique of Jacobson and Doan was used. The temperature at which the flavor appears is 76–78° C. (169–172° F.) for momentary heating and 70–72° C. (158–162° F.) for 30 minute holding.

The momentary temperature required to produce the cooked flavor is raised to 84–86° C. (183–187° F.) when 1 p.p.m. of copper was added after heating. A somewhat closer relationship was found between the cooked flavor and the sulfhydryl groups than heat labile sulfides. A lower critical temperature for cream than skim milk suggests that the proteins are associated with the fat, also these proteins were not removed by 3 washings of the cream. The retardation or prevention of oxidized flavor in cooked milk may be due to the creation of a reducing system unfavorable to oxidation or through direct combination with the metals which are oxidative catalysts. Copper is more effective in preventing or dispelling cooked flavor when added after heating. Two p.p.m. of copper has been used to dispel heated flavor in heat treated soft curd milk. E.F.G.

743. **Developments in Production and Reports in 1940.** J. F. Singleton, Assoc. Director of Marketing Service, Dairy Products, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 24. 1941.

The production and marketing of dairy products in Canada for 1940, has been conditioned greatly by the needs of the United Kingdom. There has been some diversion of milk supplies from butter to cheese and evaporated

and condensed milks. Butter prices dropped while cheese prices increased. In order to conserve supplies of cheese for the Ministry of Food, the Board has taken action to restrict exports to non-Empire countries. Production of condensed milk, evaporated milk and skim milk powder was higher than in 1939. Greater total milk production for 1941 could be used to advantage. O.F.G.

744. **Operating a Credit Bureau in the Milk Industry.** E. J. LEBOEUF, Windsor Milk Distributor's Assoc., Windsor, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 44. 1941.

The Credit Collection Agency results in a control bureau which does away with loose credits by keeping accounts from becoming inactive. The co-operative method promotes good will to all. O.F.G.

745. **Co-operation for Efficient Milk Production.** H. B. ELLENBERGER, Univ. Vermont, Burlington, Vt. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 20. 1941.

Milk can be made more cheaply when approved modern and efficient methods are practiced more generally on farms. Both co-operatives and proprietary distributors can well afford to lend a helping hand to producers, for in the long run both would profit. Many reasons exist why distributors and producers should co-operate. It is net income rather than gross income that is usually important and efficiency is the master key to better net income. Dealers and producers can accomplish more through working jointly than through independent action. O.F.G.

746. **The Determination of the Viscosity of Human Milks and the Prenatal Secretions.** GEORGE W. SCOTT BLAIR, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Biochem. Jour., *35,* No. 3: 267–271. March, 1941.

An apparatus, quite similar in principle to the Ostwald pipette, is described by which the viscosity and viscous anomalies of small (1 ml.) samples of human mammary secretions may be quickly and accurately determined.

These secretions, tested at blood heat, behave in general, surprisingly like true fluids, *i.e.*, their viscosities differ but little with varying shearing stress. V.C.S.

747. **Methods of Producing High Quality Table Cream.** O. J. SCHRENK, Bowman Dairy Co., Chicago, Ill. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 58. 1941.

All the rules and regulations laid down for the production of good milk should apply to cream. Cleaned, rinsed and sterilized equipment made of

metals that cause no off-flavors is essential to good flavor in cream. Good viscosity is essential to table cream and methods of producing a higher viscosity are described. The chief contributing factor to undesirable cream plug formation is agitation at temperatures within the churning range. Cream feathering is increased by, (1) coffee made with hard water, (2) long-time contact of coffee with the grounds, (3) high acid cream, (4) high calcium and magnesium content of cream, (5) low pasteurization temperature, (6) high homogenization pressure, (7) low homogenization temperature, and (8) single-stage homogenization. Feathering is reduced by (1) soft water, (2) short-time contact with coffee grounds, (3) low acid in cream, (4) addition of sodium citrate, (5) high pasteurization temperature, (6) low homogenization pressure, (7) high homogenization temperature, and (8) two-stage homogenization. The formation of a skim milk layer may be inhibited by high pasteurization temperature or careful homogenization. To the average consumer yellow color in the cream is an indication of richness and therefore is important. O.F.G.

748. **A Discussion of Public Relations in the Milk Industry.** H. L. GARNER, Ontario Daily Newspaper Assoc., Peterborough, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 26. 1941.

A public relations program really means a program for building public goodwill, or favorable public opinion. To sell your milk products, you must, first, win the goodwill of the public and, second, win the good opinion of the workers in your plant. Goodwill is based on good "works" and on good "words." O.F.G.

749. **Public Relations in the U. S. Milk Industry.** G. G. DIFFENBACH, Abbott's Dairies, Philadelphia, Pa. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 23. 1941.

A vast amount of work by the industry along nutritional and consumption lines is essential. An intensive type of activity must be pursued to acquaint the public with the unique position of the fluid milk distributor and the industry he represents. People must be informed more fully about the functions and operation of the dairy industry. O.F.G.

750. **Mechanical Principles and Problems of Vat Pasteurization.** A. H. RISHOI, Cherry Burrell Corp., Chicago, Ill. Dairy World, *20*, No. 1: 22. June, 1941.

This is a detailed discussion of the problem of heat transfer in vats used for pasteurizing milk products particularly with reference to conductivity of the material separating the heating or cooling medium and the fluid being heated or cooled, the temperature gradient and the importance of agitation

on either side of the material through which the heat flows. The following conductivity table is presented:

Material	Temperature °F.	Conductivity
Aluminum	64	0.504
Brass	64	0.260
Copper	64	0.918
German silver	32	0.700
Iron	64	0.161
Steel	64	0.115
Stainless steel	212	0.039
Nickel	64	0.142
Tin	64	0.155
Glass	68	0.002
Water	68	0.00143
Air	32	0.0000568

F.J.D

751. **Pasteurization of Modified Milk Products.** K. G. Weckel, Univ. Wisconsin, Madison, Wis. Dairy World, *20*, No. 2: 17. July, 1941.

Pasteurization treatments for milk "by-products" differ from that for milk since body or viscosity, texture, flavor, solution of ingredients, color, bacteriological effects, etc., are often extremely important considerations, whereas with milk the main concern is to render the product free of pathogens. The author discusses the pasteurization or heat treatment problem involved in the manufacture of buttermilk, cultured cream, chocolate milk, pasteurized cream, homogenized milk, cheese spreads, ice cream mix and some less important products. F.J.D.

752. **Oxygen Constant Variants.** E. S. Guthrie, Paul F. Sharp and David B. Hand, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *2*, No. 6: 131, 132. June, 1940.

Milk absorbs from 3.84 to 9.74 p.p.m. of oxygen during hand milking but contains none while in the udder. It is absorbed slowly when the surface is quiet, is driven out during vat pasteurization, and reabsorbed during cooling and bottling. If deaerated it may be bottled without additional air by admission to the bottom of the bottle through a tube, taking care to cause a minimum of agitation. P.S.L.

753. **Testing Pasteurized Cream.** Herbert Jenkins. Amer. Milk Rev., *3*, No. 3: 58–60. March, 1941.

Seeking to cut the time required for plate counts of pasteurized cream, many tests using resazurin dye were run, the time required being 6 to 7 hours. Those creams having a reduction time of 6 to 7 hours, when plated, had a plate count of less than 40,000 bacteria, the average being 17,000. Such cream occasioned no complaints from customers. P.S.L.

754. **Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method.** A. W. TURNER, University of Illinois, Urbana, Ill. Soc. Expt. Biol. and Med. Proc., *46:* 593. 1941.

An investigation was made of the characteristics of enzyme-treated milk. The enzyme treatment was carried out by adding one part of pancreatic concentrate to 10 to 15 thousand parts of cold raw whole cow milk followed by immediate pasteurization. Enzyme-treated milk contained more 70 per cent alcohol-soluble protein and proteose peptone nitrogen but only slightly more amino nitrogen than ordinary pasteurized milk. Casein prepared from enzyme-treated skim milk was more soluble in 70 per cent alcohol than was casein prepared from pasteurized skim milk. R.P.R.

755. **Are You Encouraging the Love Life of a Fly?** ANONYMOUS. Milk Dealer, *30,* No. 8: 76–80. May 1941.

A discussion is given on how to control the fly in dairy plants. The article is summarized as follows: The best arrangement for controlling the fly menace, it would seem, is first to clean up and keep clean the premises; second, screen all doors and windows; third, supplement screens with electric screens to kill flies seeking admission; fourth, use a good power spraying system to get rid of the flies which get into the plant. C.J.B.

756. **The Contribution of Industrial Milk Service to National Defense.** JAMES R. HUDSON, Baker-Stuber Dairy, Peoria, Ill. Milk Dealer, *30,* No. 8: 116–121. May 1941.

A discussion is given of how the dairy industry can contribute to national defense by supplying factory workers with enough milk to keep up their efficiency, reduce absenteeism, and maintain their morale. C.J.B.

757. **The Instantaneous Heat Treatment of Milk.** G. C. SUPPLEE AND O. G. JENSEN, Borden Co., Bainbridge, N. Y. Jour. Milk Technol., *4,* No. 1: 5. 1941. (Also published in the Annual Proceedings of the New York State Assoc. of Dairy and Milk Insp., 1940.)

Using a flowing film electric pasteurizer the authors studied the effect of momentary exposure periods at various temperatures on bactericidal effectiveness, flavor, cream line and phosphatase test.

Exposure periods as short as 0.8 second between the lethal temperature range of 145° F.–185° F. were found effective in bactericidal reduction. No automatic controls were used on the equipment either to regulate the temperature or the flow of current. Variations in temperature from the average operating level did not exceed about 3°, and it was believed that the major variations may have been due to surges in the feed line voltage.

It was found that 76 per cent of the milks exposed to 180° F. and above showed a percentage reduction in bacterial count of over 99 per cent.

Data was obtained on the phosphatase test using Gilcreas and Davis' modification for average or mean temperatures of 163°, 173°, 177°, 181°, and 186° F.

At 163° F. all samples were classified as grossly underpasteurized, showing values of 0.15 and above. At 173° F. they varied from 0.03 to 0.15. At 177° F. they varied from 0.00 to 0.09; about 70 per cent of the samples were classified as satisfactorily pasteurized and the remainder as slightly underpasteurized. At 181° F. and above they were all classified as satisfactorily pasteurized having values of 0.04 or less.

The characteristic heated milk flavor was practically undetectable at operating temperature of 185–186° F. Improvement in the flavor was even noted at operating temperature of 185° F. and lower. At 190° F. the heated flavor was detected by experts, but was not as pronounced as is frequently observed in milk pasteurized by usual methods.

Reduction in creaming ability when compared to the raw milks ranged from 6 to 20 per cent through the temperature range of 160° F. to about 180° F. Creaming ability was destroyed most rapidly per degree increase in temperature through the 185–190° F. range. L.H.B.

758. A Simplified Procedure for Laboratory Examination of Raw Milk Supplies. R. P. Meyer and J. A. Pence, Sealtest, Inc., Baltimore, Md. Jour. Milk Technol., *4*, No. 1: 18. 1941.

For the purpose of testing producers' milk to find if it contains thermoduric organisms, the use of the loop measurement, oval tube technique saves time and material in making the laboratory pasteurization tests.

Test briefly is as follows:

Pasteurize 5-cc. samples of milk in screw-capped vial at 143° F. for 30 minutes, in a constant temperature bath, cool, shake vigorously 50 times. With standard loop needle (0.001 or 0.01 cc.) transfer loopful of milk to an oval culture tube (size 152 mm. in length and 23 mm. by 11.5 mm. in diameter) containing approximately 4 cc. of sterile melted T.G.E. agar cooled to 45° C. Mix contents by swinging tube through a small arc for about 5 seconds. Tube is then laid on table with open end raised about ⅛ inch so tube is slanted to permit agar to flow to a point 2.5 to 3 inches from bottom of tube. Allow agar to harden. Place tubes in special wire rack in horizontal position with agar adhering to upper side of tube. Incubate for 48 hours at 37° C. Count colonies in usual manner by placing tubes over a well lighted colony counter. This method is reported to save one-half the time, uses only ⅓ as much agar, and requires no dilution blanks and pipettes.

Results checked very closely with standard agar plate count using T.G.E.M. agar. L.H.B.

759. **To What Extent Should Bacterial Counts of Milk be Given Publicity.** C. C. Prouty, Agr. Expt. Sta., Washington State College, Pullman, Wash. Jour. Milk Technol., *4,* No. 1: 32. 1941.

Bacterial counts should not be given publicity on the ground that the milk consuming public is not aware of the limitations of making bacterial counts, and therefore, not qualified to interpret them properly in relation to the sanitary quality of the product.

Equal ratings should be given to all samples falling into the same count bracket.

A discussion of the paper by M. E. Parker, of the Beatrice Creamery Company, Chicago, Illinois, is also given. L.H.B.

760. **"Approved Milk" for New York City in Place of Grade A and Grade B.** J. L. Rice and Sol Pincus, N. Y. City Dept. Health. Jour. Milk Technol., *4,* No. 1: 38. 1941.

A history of New York City's milk regulations are given reviewing the events in the development of milk control which leads up to the latest step of eliminating A and B grades and replacing with "Approved Milk."

The standards for the "approved milk" are stricter than formerly called to grade B milk which was the bulk of their supply. A chart showing the former requirements for grade A and B is given comparing the standards with those of "approved milk."

Advantages expected to be gained by simplification of grading system are:

1. It will be possible to concentrate all energies upon improving general supply without regard to grading.

2. With the elimination of dual grading the consideration of grade A as the only safe milk was removed as was the inferiority implication given grade B. Confidence of public in their milk supply will be encouraged.

3. Sanitary control will be simplified and the industry will be enabled to eliminate some plant duplication, where formerly they were required to maintain separate equipment for handling grades A and B. L.H.B.

761. **Report of Committee on Applied Laboratory Methods.** T. H. Butterworth, San Antonio, Texas. Jour. Milk Technol., *4,* No. 1: 44. 1941.

The committee presents a program of work for the coming year. At least one member of the committee is actively interested in one or more of the subjects which are as follows:

1. A study of requirement recommendations for officially certified milk and milk products analysis laboratories.

2. A study of the relationship of laboratory tests to field inspection work and an evalution of the emphasis to be placed on each.

3. A study of the use of the reductase test in controlling raw-to-plant milk supplies.

4. A study of the tentative 32° C. temperature requirements for milk and milk produce incubation.

5. A study of the numerical bacterial content of city supplies of raw-to-plant milk and the best tests for estimating same.

6. By means of a questionnaire to the industry and control officials, a study of the present usefulness of the phosphatase test and the most valuable modification for general use. L.H.B.

762. **Some Practical Applications of Milk Technology.** E. EUGENE CHADWICK, Acting City Sanitarian, Astoria, Ore. Jour. Milk Technol., *4*, No. 1: 45. 1941.

A discussion is given of how two cities in Oregon improved the sanitary conditions of their milk supplies. Milk consumption has increased from 0.7 pint per person to 1.3 pints. L.H.B.

PHYSIOLOGY

763. **Growth of the Lobule-Alveolar System of the Mammary Gland with Pregneninolone.** JOHN P. MIXNER AND CHARLES W. TURNER, Univ. of Missouri, Columbia. Soc. Expt. Biol. and Med. Proc., *47:* 453. 1941.

The injection of pregneninolone either alone or in conjunction with estrone into spayed virgin mice caused the development of the lobule-alveolar system of their mammary glands a property similar to progesterone. Injected estrone enhanced the activity of the pregneninolone about 5 times while under similar conditions progesterone was found to be about twice as effective. R.P.R.

764. **Death of Embryos in Guinea Pigs on Diets Low in Vitamin E.** ALWIN M. PAPPENHEIMER AND MARIANNE GOETTSCH, Columbia Univ., New York. Soc. Expt. Biol. and Med. Proc., *47:* 268. 1941.

A diet low in vitamin E supplemented by 5 to 10 mg. of alpha-tocopherol protected 5 guinea pigs against muscular dystrophy but the amount of supplied vitamin was not adequate to insure successful pregnancy. Three pigs receiving 5 mg. of alpha-tocopheral had resorption at about 30 days and of 2 pigs receiving 10 mg. one gave birth to a living young, followed by resorption (initial fertility) and the other one went beyond mid-term, dying on the 47th day. R.P.R.

765. **Effect of Desoxycorticosterone on Pituitary and Lactogen Content.** C. W. TURNER AND JOSEPH MEITES, Univ. of Missouri, Columbia. Endocrinology, *47:* 232. 1941.

Three groups of female and 2 groups of male guinea pigs were injected

with 7 to 20 mg. of desoxycorticosterone acetate over a period of 10 to 20 days to determine its effect on pituitary lactogen content and pituitary weight. The treatment produced a significant increase in pituitary weight but the lactogen content was not altered. R.P.R.

766. **Biennial Reviews of the Progress of Dairy Science. Section A. Physiology of Dairy Cattle. I. Reproduction and Lactation.** Jour. Dairy Res., *12*, No. 1: 78–107. 1941.

A review of recent literature under the subheadings: Hormones, Biochemical Aspects, Anatomical Aspects, Clinical Chemistry, Climatic and Other Factors Affecting Milk Secretion is given with 208 references.
S.T.C.

767. **Some Experiments on the Chemical Enrichment of Cows' Milk by the Administration of Diethylstilbestrol and Its Dipropionate.** S. J. Folley, H. M. Scott Watson and A. C. Bottomley, Natl. Inst. Res. Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 1–17. 1941.

The results secured were extremely variable as is shown by the following summary:

1. Diethylstilbestrol administered orally to a Shorthorn cow had no marked effect on milk yield or composition.

2. A series of injections of the dipropionate in oily solution led to a slight rise in non-fatty solids in the same cow as in (1). Single larger injections were followed by a rise in milk solids accompanied by a rapid fall in milk yield in two other Shorthorns.

3. Inunction with an ointment containing the dipropionate led to a marked increase in milk solids in a Shorthorn cow, with no change in milk yield. The effect subsided rapidly when treatment was stopped. No significant effects were produced by similar treatment of four pregnant British Friesians; on increasing the dose two of these aborted. A Guernsey cow showed a slight increase in non-fatty solids and a slight, but temporary, fall in milk yield.

4. Subcutaneous implantation of crystalline diethylstilbestrol led to a striking and prolonged increase in milk solids, with no fall in milk yield, in a Shorthorn cow.

5. Subcutaneous injection of an aqueous suspension of diethylstilbestrol (1 g.) was equally successful when applied to the same cow as 4, but in the next lactation. In three Ayrshires the increase in solids was accompanied by an appreciable decline in milk yield. A Shorthorn receiving 375 mg. showed a temporary rise in solids, while one receiving 225 mg. showed no effect.

6. In all cases where milk yields declined the milk solids percentage rose, but the converse did not hold. Hence, the threshold dose for inhibition is apparently higher than for enrichment.

7. The threshold doses may depend on the breed; the most successful results were obtained with Shorthorns.

8. Treated cows may be difficult to get in calf subsequently, especially those treated twice.

9. Administration of large doses of diethylstilbestrol to cows in advanced pregnancy results in abortion.

10. The enrichment of the milk in favorable cases represented a true increase in the yield of solids secreted, and not merely a concentration due to reduced secretion of water. S.T.C.

768. **Comparison of Assay Methods Using International Standard Lactogen.** J. MEITES, A. J. BERGMAN AND C. W. TURNER, Univ. Missouri, Columbia. Endocrinology, *28:* 707. 1941.

Three methods of assay of International Standard lactogen were compared, all assays based upon a 50 per cent minimum crop gland proliferation response in 20 common pigeons weighing 300 ± 40 gm. The subcutaneous route of administration required 0.1 mg. of the International Standard to equal the International Unit. The shallow intrapectoral method required 1.25 International Standard Units and the intradermal (micro) method required 1/160 of an International Unit. In connection with the intradermal method it was shown that a 2, 3 and 5 fold difference in injection volume containing the same amount of hormone caused no change in effectiveness of the crop gland responses. R.P.R.

769. **Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat.** HERBERT M. EVANS, MIRIAM E. SIMPSON AND WILLIAM R. LYONS, Dept. Anatomy, Univ. California, Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., *46:* 586. 1941.

Experiments were conducted which demonstrated that the lactogenic hormone was the only pituitary preparation which would stimulate the production of progestin by either normally occurring or artificially induced lutein tissue in the rat. R.P.R.

770. **Local Responses of the "Sexual Skin" and Mammary Glands of Monkeys to Cutaneous Applications of Estrogen.** T. L. CHAMBERLIN, W. U. GARDNER AND E. ALLEN, Yale University, New Haven, Conn. Endocrinoloy, *28:* 753. 1941.

Small doses of estrogen in alcohol applied cutaneously induced local responses in the sexual skin of immature female monkeys. One to 3 gamma of estrone in alcohol daily for 8 to 12 days on one side produced a unilateral reaction while alcohol alone on the other side served as a control. Similar

treatment on one breast of young male monkeys induced considerably more growth in both nipple and glandular tissue of that side. The other mammary gland showed slight growth. R.P.R.

771. **Rumen Synthesis of the Vitamin B Complex on Natural Rations.** M. J. Wegner, A. N. Booth, C. A. Elvehjem and E. B. Hart, Univ. Wisconsin, Madison, Wis. Soc. Expt. Biol. Med. Proc., *47:* 90. 1941.

Six members of the vitamin B complex were determined in the rumen ingesta of a heifer fed a ration composed of natural feeds. In most cases higher values were found in the rumen ingesta than in the ration fed. With the exception of riboflavin, variation of the amount of urea or protein in the grain mixture of the ration had little if any effect on the vitamin content of the ingesta. The authors were of the opinion that the increase in B vitamins in the ingesta as contrasted with the ration fed was due to a synthesis and not to a concentration effect. R.P.R.

772. **Inability of Desoxycorticosterone to Maintain Lactation.** Robert Gaunt, Washington Square College of Arts and Science, New York Univ., New York City. Soc. Expt. Biol. and Med. Proc., *47:* 28. 1941.

A study was made of the effect of desoxycorticosterone acetate on the lactation of rats adrenalectomized within 24 hours after parturition. The necessity of adrenal cortical secretions for the support of lactation in rats was confirmed. Desoxycorticosterone acetate, unlike adrenal cortical extract, was of no benefit at all in correcting this deficiency and might have further depressed lactation. R.P.R.

773. **The Interrelation of Oxidative and Glycolytic Processes as Sources of Energy for Bull Spermatozoa.** Henry A. Lardy and Paul H. Phillips, Univ. Wisconsin, Madison, Wis. Amer. Jour. Physiol., *133:* 602–609. 1941.

In spermatozoa the energy requirement for the maintenance of their vital activity can be obtained from the oxidation of intracellular phospholipids or from glycolytic processes. When sugars are available to the spermatozoa the energy obtained from their breakdown to lactic acid lessens the demand on the intracellular lipids. D.E.

774. **Brunsterzeugung bei Schafen mit Geschlechtshormonen.** H. M. August, Office of Animal Health, Breslau. Züchtungskunde, *16,* No. 2: 41–60. 1941.

In an attempt to cause sheep to come in heat in the spring so that fall lambs could be produced, 4,281 ewes in 28 flocks in German Silesia in the spring of 1938 were given intravenous injections of follicular hormone,

either alone or together with Prolan. Of the treated ewes 72.5 per cent accepted service and showed other signs of estrus and 43.1 per cent, (59.5 per cent of those which were served) produced lambs. Results appeared to be the same whether the folliculin was accompanied by Prolan or not. The methods used are not yet satisfactory for widespread practical application. The prospects for success are too uncertain, good results being obtained in some flocks but completely unsatisfactory results in others. Satisfactory explanations for these discrepancies were not found, but the problem is of enough practical importance and the present results are promising enough to deserve further investigation. J.L.L.

MISCELLANEOUS

775. **Selling and Advertising Dairy Products. 1. "Let the Taste Tell and Sell."** H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 56. 1941.

Since nothing the dairy can say or write or picture about its products equals a taste of the product itself, sampling is an important sales aid to the progressive dairyman. The salesman should give the housewife a sample of all his products. Advice to the salesman is, (1) arouse interest, (2) cut down length of sales story, (3) arrange the sales story in the best possible sequence, (4) close with a bang. Let taste, not a description of taste, do your selling. O.F.G.

776. **Selling and Adveristing Dairy Products. 3. "Selling Through the Kiddies."** H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., *20,* No. 3: 46. 1941.

The author suggested that an excellent approach to dairy sales is through children. Organize children's clubs, sports and contests, birthday clubs and write the children friendly letters, is his advice after having tried them. Other means of approach are through child talent radio programs and educational school films. O.F.G.

777. **Utilization of Skim Milk and Whey in Precooked Dried Soup.** G. A. RAMSDELL AND B. H. WEBB, Div. Dairy Res. Labs., Bur. Dairy Indus., Washington, D. C. Food Res., *6,* No. 3: 265. May–June, 1941.

Skim milk and whey were found to improve both the flavor and the body of spray dried soup mixtures when used in quantities up to 25 per cent of the weight of the dry mix. F.J.D.

778. **Corrosion of 18–8 Stainless Steel in Sodium Chloride Solutions.** H. H. UHLIG AND M. C. MORRILL, Mass. Inst. of Tech., Cambridge, Mass. Indus. and Engin. Chem., *33,* No. 7, 875–880. 1941.

A detailed study is reported of the effect of temperature, concentration,

and pH of aerated sodium chloride solutions, on the nature and rate of corrosion for 24 hour periods of 18-8 stainless steel. Corrosion increases sharply with temperature, reaching a maximum with 1 per cent to 10 per cent NaCl solutions at 90° C. and above but at the boiling point corrosion decreases to nearly zero due to lack of dissolved oxygen. Maximum corrosion occurred at 90° C. with 4 per cent NaCl solution. Maximum pit penetration in 4 per cent NaCl at 90° C. was at pH 6 to 7 and this fell to a minimum at pH 2.9 to 4.5 with a sharp increase in corrosion below pH 2.9. There was a drop in corrosion above pH 7 to a minimum at pH 12. B.H.W.

779. **Fleet Maintenance.** ANONYMOUS. Milk Dealer, *30*, No. 8: 38–39, 87–90. May, 1941.

A description is given of the efficient system of fleet maintenance as carried on by the Alfar Creamery Company in West Palm Beach, Florida. To get an accurate account of what it costs to operate the trucks for the benefit of figuring cost of delivery, as well as the garage department expense, an analysis is made of each truck on separate cards. Samples of the cards used are presented. C.J.B.

780. **An Apparatus for Comparison of Foaming Properties of Soaps and Detergents.** JOHN ROSS AND GILBERT D. MILES, Colgate Palmolive Peet Co., Jersey City, N. J. Oil and Soap, *18*, No. 5: 99. May, 1941.

A simple apparatus and procedure is described for measuring the foaming properties of soaps and detergents. By this method the relative stability of foams is compared by measuring the effect of an arbitrary standard destructive mechanism acting upon the volume of foam during production under standard conditions and protected from adventitious destructive forces. V.C.S.

781. **The Use of Neoprene in Refrigeration Equipment.** H. LOGAN LAWRENCE, E. I. duPont de Nemours and Co., Wilmington, Del. Refrig. Engin., *41*, No. 6: 404. 1941.

Details of the use of neoprene, a substitute for rubber, in small type refrigeration equipment, it being little effected by the usual refrigerants. It is an excellent rotary sealing material and is especially efficient as an insulating material for motor windings of sealed-in units even for motor sizes up to 7½ h.p. It is also used for refrigerator door gaskets and machine gaskets. While the price of a neoprene gasket is 25 per cent greater than that of a high-grade rubber gasket, the increased service life much more than offsets the higher first cost. L.M.D.

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PRESERVATION OF BOVINE SPERMATOZOA IN YOLK-CITRATE DILUENT AND FIELD RESULTS FROM ITS USE

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INTRODUCTION

Scherstén (11) found appreciable quantities of citrates in the sexual gland secretions of man and animals and stated that a considerable portion of the buffer capacity of the semen was due to citrates. Others, including Slowtzoff (12), Huggins and Johnson (5), and Goldblatt (3), working with human beings, and McKenzie and co-workers (6), working with the boar, have analyzed semen, and their work shows that it contains considerable quantities of phosphates and carbonates. Willett (14) recently has determined the buffer coefficient curves for representative samples of the semen of the bull, the stallion, man, and the dog. The peaks of buffer capacity of bull semen coincide with the pH levels at which citrates, phosphates, and carbonates would be effective as buffers.

Scherstén (11) found that addition of sodium citrate to a Ringer-phosphate solution at the rate of 30 to 60 mg. per 100 cc. increased the longevity of spermatozoa suspended therein. Earlier Gray (4) had used sodium citrate to disperse spermatozoa agglutinated by metallic ions. However, Fleig (2) and Dubincik (1) have reported that citrate anion had a deleterious effect on sperm.

In light of these several investigations it was thought worth-while to develop a buffer mixture for the preservation of bull spermatozoa which would correspond closely to the buffers of semen to be used with egg yolk in place of the phosphate suggested by Phillips (8) and Phillips and Lardy (9), and which had given such splendid results in investigations by us (15).

LABORATORY STUDIES

An M/15 solution of sodium citrate and an M/15 solution of potassium di-hydrogen phosphate were added to egg yolk in such amounts as to make the resulting mixture contain about equal parts of egg yolk and buffer with

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the pH adjusted at 6.75. When first used it was noted that the new citrate buffer dispersed the fat globules and other materials in the yolk so that the resulting mixture was clear in appearance. When using the microscope to examine a sample of semen diluted with this mixture one could readily discern the individual sperm; on the other hand, when using the yolk-phosphate diluent it was necessary to further dilute the mixture with a clear diluter before the individual sperm could be distinguished.

Since this property was lost when the proportion of citrate was reduced by a small amount it was decided to use only enough potassium di-hydrogen phosphate as was compatible with satisfactory storage. Investigation showed that the phosphate buffer was not necessary for satisfactory results in the storage of semen when egg yolk was also used. Consequently, in the investigations herein reported the yolk-citrate diluent was composed of equal parts of fresh egg yolk and an M/15 solution of sodium citrate. Four or five parts of this diluent were added to one part of semen for the storage studies.

Comparisons were then undertaken to determine the relative value of the yolk-citrate diluent and the yolk-phosphate diluent in preserving the motility of spermatozoa under standard storage conditions. All the semen used in this investigation was collected from the dairy bulls in the Cornell University herd with the new-type artificial vagina (10). The semen was divided into equal portions immediately after collection. One-half was diluted at the rate of 1 part of semen to 4 or 5 parts of the yolk-citrate diluent. The other half was diluted at the same rate with yolk-phosphate dilutent made after the directions of Phillips and Lardy (9).

The diluted samples were divided into 0.5 cc. portions and placed in small culture tubes. These tubes were then wrapped individually with paper and put into larger test tubes. The larger tubes were then placed in controlled-temperature water baths for cooling. Cooling was accomplished by changing the tubes from one bath to another at definite intervals. In these studies the semen was cooled from room temperature to the storage temperature of 5° C. at the rate of 5° C. per 10 minutes. After storage the samples were warmed at the rate of 10° C. per 10 minutes. Willett (14) has shown that, while one must carefully control the rate of cooling of semen for best results, the rate of warming the sample is apparently of little importance.

Each sample of semen was examined for motility at 37° C. in a microscope stage incubator immediately after collection and after 2, 4, 6, 8, and 10 days of storage. With the yolk-phosphate diluent it was necessary to further dilute the samples with SGC–2 diluter (Milovanov (7)) so that the spermatozoa might be readily seen. Studies of the buffer coefficient curves of this diluter showed that it possessed no appreciable buffer capacity and did not influence the pH of the stored semen plus yolk-diluent when added

to it. Estimations of motility were made on the basis of the proportion of actively motile spermatozoa and were expressed as per cent to the nearest unit of 10. Willett (14) has shown that this method of estimation of motility gives highly repeatable results.

RESULTS OF LABORATORY STUDIES

In table 1 is presented a summary of the studies comparing the motility of the spermatozoa during storage in the citrate and phosphate diluents. By the analysis of variance technique (13), no significant differences were

TABLE 1

Comparison of motility of spermatozoa during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	Motility means			Probabilities for differences between means
		Before storage	After storage		
			Phosphate	Citrate	
days	*No.*	%	%	%	
2	19	71	47	50	> .05
4	19	72	38	40	> .05
6	17	72	26	35	< .01
8	19	72	23	32	< .01
10	16	72	13	22	< .01

detected after 2 and 4 days of storage. After longer intervals of storage the differences were highly significant in favor of the citrate diluent. In addition, it was noted that, especially after 6 and 8 days storage, the spermatozoa were more active and many more showed progressive motility in the citrate than in the phosphate diluent.

In table 2 is presented a summary of the pH values of the semen-yolk-buffer mixtures before and after storage. It can be readily seen that there was practically no difference between the average pH values of the two mixtures either before or after storage for different intervals.

TABLE 2

Comparison of pH of semen during storage in yolk-phosphate and yolk-citrate diluents at 5° C. and without mineral oil

Time stored	Sample pairs	pH means			
		Before storage		After storage	
		Phosphate	Citrate	Phosphate	Citrate
days	*No.*	*pH*	*pH*	*pH*	*pH*
2	18	6.74	6.71	6.62	6.64
4	19	6.74	6.71	6.55	6.54
6	17	6.73	6.70	6.54	6.50
8	19	6.73	6.70	6.50	6.45
10	15	6.72	6.70	6.45	6.44

Although the average pH values of the mixtures before storage were about the same, the values for the semen in the citrate buffer were more variable than those for the phosphate buffers, for their coefficients of variation were 1.12 per cent and 0.73 per cent, respectively. This can be explained by the lower buffer capacity of the citrate diluent at these pH levels, with the result that the pH of the semen, which is quite variable, would have a greater influence on the pH of the citrate than on the phosphate diluent.

FIELD STUDIES AND RESULTS

Since the citrate buffer appeared to excel the phosphate buffer when used with egg yolk for the preservation of semen during extended storage, an experiment was designed to compare the fertility of spermatozoa stored for various intervals in the two diluents. This experiment was undertaken in cooperation with the Seneca (Seneca County, New York) Cooperative Cattle Breeders' Association, Inc., and all of the inseminations were carried out by H. K. Fuller.

The semen was collected and handled in a manner already described (15). Alternate ejaculates from each bull were preserved in the citrate and the phosphate diluents and then used for breeding after various intervals of storage. Semen from five bulls was used and the results for each bull are reported separately in table 3. Bulls number I and II are Holsteins, the others are Guernseys.

Save for the first twenty-four-hour period, the use of the citrate and phosphate diluents gave comparable results until the fifth day when the citrate gave slightly better results. However, this latter difference was not significant, nor were any of the other differences between the results obtained with the yolk-citrate and yolk-phosphate diluents significant when analysed with chi-square in a two by two table. Furthermore, when the values for all of the time intervals were totalled, the numbers of services required per conception with the semen preserved in the two diluents were practically identical. As far as these fertility studies go they tend to bear out the results of the storage studies which showed no significant differences until the sixth day and after. If the fertility studies had been extended over a longer period of storage the superiority of the citrate diluent which was indicated from the results of inseminations during the fifth day might have been established as real.

SUMMARY

1. An M/15 solution of sodium citrate mixed in equal amounts with fresh egg yolk produced a diluent which dispersed the fat globules and other material in the yolk so that when semen was diluted with it the individual spermatozoa could be readily seen upon microscopic examination.

2. The yolk-citrate diluent and the yolk-phosphate diluent were ap-

TABLE 3

Breeding results in the Seneca Cooperative Cattle Breeders' Association when comparing the yolk-phosphate and yolk-citrate diluents

Bull	Age of semen used (hours)														Services per conception
	0–24		24–48		48–72		72–96		96–120		120–144		Totals		
	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	Serv.	Conc.	
Yolk-phosphate diluent															
I	10	6	8	6	14	7	6	4	11	7	2	1	51	31	1.65
II	1	1	9	6	4	3	6	4	6	3	9	4	35	21	1.67
III	5	5	9	5	10	8	8	7	4	2	......	......	36	27	1.33
IV	6	4	10	7	7	4	5	4	2	2	......	......	30	21	1.43
V	2	1	7	6	10	6	11	8	9	5	2	0	41	26	1.58
Totals	24	17	43	30	45	28	36	27	32	19	13	5	193	126	
Serv. per conc.	1.41		1.43		1.61		1.33		1.68		2.60		1.53		
Yolk-citrate diluent															
I	......	......	16	13	14	8	16	10	1	0	......	......	47	31	1.52
II	4	4	5	3	13	8	16	11	9	6	5	3	52	35	1.49
III	5	2	11	8	4	1	2	2	1	0	......	......	23	13	1.77
IV	2	0	15	10	5	4	4	4	4	1	......	......	30	19	1.58
V	1	1	8	7	8	5	5	3	4	4	......	......	26	20	1.30
Totals	12	7	55	41	44	26	43	30	19	11	5	3	178	118	
Serv. per conc.	1.71		1.34		1.69		1.43		1.73		1.67		1.51		

parently equal as preservatives of the motility of spermatozoa which were stored in them under standard conditions for two and four days.

3. The yolk-citrate diluent was superior to the yolk-phosphate diluent for the preservation of motility when semen was stored six days or more.

4. In actual insemination tests with semen stored up to 5 days no significant difference in fertility was found between the semen stored in the yolk-citrate and the yolk-phosphate diluents.

5. The results of the fertility studies tend to bear out the results of laboratory storage studies on the maintenance of motility under standard conditions.

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THE BACTERIOLOGY OF BULL SEMEN

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Practically all workers in the rapidly expanding field of artificial insemination stress the necessity of bacteriological control. It therefore seems desirable to know definitely what measure of bacteriological control is necessary or desirable in the collection, handling, and storage of semen. In addition, information on the types and numbers of bacteria present under varying conditions of collection and storage and their possible relation to infection in the female genital tract would be useful. Attention was earlier called to these facts by Salisbury, Willett and Gunsalus (14).

Most of the available information on the bacteria of the reproductive tract or semen of bulls deals either with the incidence and spread of disease, such as Bang's disease, or with possible causes of sterility of bulls with poor breeding records. Little attention has been given to the subject in relation to the preservation of semen and to artificial insemination.

Various methods have been used to obtain semen for bacteriological study. Webster (17), Gilman (4), and Williams and Kingsbury (19) recovered semen from the vaginas of cows immediately after normal service. The vaginas and sheaths having previously been douched and disinfected as suggested by Williams. Gilman, and Williams and Kingsbury reported finding micrococci, hemolytic and non-hemolytic streptococci, coliform organisms and *Brucella abortus*. They concluded that the genital tract and semen of normal bulls contained few, if any, bacteria, but that sterile bulls or those of diminished fertility usually contained large numbers. Webster reported that on culture normal semen yielded diphtheroids and micrococci, while bulls from herds in which enzootic sterility was present produced semen which contained, in addition, many alpha hemolytic streptococci. As suggested by Gilman, bacteriological studies on semen obtained by this technique are not entirely satisfactory due to possible contamination from the vagina.

Gilman (3) also studied the genital tracts of bulls shortly after slaughter and reported the presence of *Pseudomonas pyocyaneus* and unidentified rods in addition to the micrococci, streptococci, and coliform organisms. His findings also indicated that the genital tracts of normal healthy bulls were either free from bacteria or contained very low numbers, whereas impotent bulls harbored large numbers of organisms which were undoubtedly ejaculated with the semen. Gilman considered infection to be the greatest single

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cause of functional and anatomical changes resulting in varying degrees of impotency.

Hatziolos (7) collected semen by use of the artificial vagina and attempted to obtain the semen as free from bacterial contamination as possible. The bacteria, which he found in every ejaculate, consisted mainly of coliform organisms, the proteus, and pseudomonas groups, cocci and spore forming rods.

That these types of bacteria can be responsible for metritis, cervicitis, sterility, or abortion in cows has been indicated by the observations of Lucet (9), Rosenow and Davis (12), Beaver, Boyd, and Fitch (1), Udall, Cushing and Fincher (16), and others.

Semen is a rather good medium for the growth of bacteria. Probably the first to report such an observation was Spallanzani (15) who in 1785 observed that the sperm from the terrestrial fetid toad soon became putrid. He attributed the diminishment of fertility of the sperm during storage to this putrefaction. Roemmele (11) noticed bacteria in semen stored at room temperature and at 9° C., and attributed the death of the sperm to the accumulation of the metabolic products of the bacteria. Hammond (6) observed bacterial growth in samples stored at 10° C., but found no greater incidence of infection in female rabbits inseminated with semen in which large numbers of bacteria had developed than with samples where fewer organism had grown due to a lower storage temperature. Krzyszkowsky and Pawlow (8) also mentioned that bacteria grew rapidly in semen, especially a small motile bacillus which formed a blue-green pigment (probably *Pseudomonas pyocyaneus*), though no definite data could be found to indicate whether bacteria have a direct detrimental effect on spermatozoa. Krzyszkowsky and Pawlow (8) thought that the products of metabolism of bacteria influenced the spermatozoon life. Gunn (5) on the other hand could detect no influence of bacteria on the survival of spermatozoa stored at low temperatures, (4° C.).

METHODS

The semen used in these studies was collected with the new-type artificial vagina (Salisbury and Willett (13)). The rubber liner of this device was sterilized by flushing with alcohol and drying before use. All glassware with which the semen came in contact was sterilized with dry heat. The mineral oil, petrolatum used as a lubricant, Milovanov's (10) SGC–2 diluent and phosphate buffers were sterilized by autoclaving.

The numbers of bacteria were determined by plate counts on blood agar containing 2 per cent sterile defibrinated horse blood. All plates were incubated 4 days at 37° C. This incubation period was selected in order to facilitate counting when slow growing diphtheroid organisms were present. The relative numbers of coliform bacteria were determined by serial dilutions in Durham tubes of glucose broth.

EXPERIMENTAL

Numbers and Predominating Types of Bacteria. The semen used in these studies was obtained from bulls in the University dairy herd and from bulls in the Central New York Artificial Breeders' Cooperative at Syracuse. The bulls at the University were used in the breeding program of the herd more or less frequently and bred the cows naturally. Since these bulls had access to exercise lots which often were muddy, their underlines and sheaths were frequently dirty. Unless the bulls at Syracuse had been brought into the herd only a short time before the samples were obtained they had not bred cows naturally for considerable periods of time, unless they had accidentally bred a cow in the breeding rack while the artificial vagina was being used. In addition, they were confined to stalls during the major portion of the day and were out-of-doors only while being exercised on a mechanical exerciser. As a result these bulls were clean about the underline and sheath.

TABLE 1

Number and kinds of bacteria found in fresh bull semen

Bull	Number ejaculates	Bacteria per cc. of semen (in thousands)		Predominating kinds
		Average	Range	
1	1	650	....	Pseudomonas, *E. coli*
2	2	7	1– 13	Diphtheroids
4	1	20	....	....
6	1	15	....	Diphtheroids
7	2	2	1– 2	" , Staphylococci
8	1	750	....	Pseudomonas, *E. coli*
9	1	30	....	Diphtheroids
21	1	90	....	" , Bacilli
22	2	6	1– 12	"
23	1	1	....	....
24	1	4,300	....	Diphtheroids, Pseudomonas, *E. coli*
26	1	50	....	" , Staphylococci, *E. coli*
D	5	1,270	13– 4,900	"
E (1939)	6	4,600	290–22,000	" , (some hemolytic)
E (1940)	6	230	10– 750	" , Pseudomonas
F	2	120	5– 230	....
H	4	6,500	10–20,000	Diphtheroids
I	2	260	130– 390	....
J	1	1	....	Pseudomonas
K	2	720	690– 750	....

The data in table 1 indicate the numbers and predominating kinds of bacteria found in fresh ejaculates when no precautions were taken in cleaning the underline or sheath of the bulls prior to collection. Of the 12 bulls in the artificial breeding herd (represented by numbers in table 1), two-thirds gave plate counts of fifty thousand bacteria per cc. or less and only 3 gave plate counts of over one hundred thousand. One of these (number 24), which gave semen with over four million bacteria per cc., had not been

used previously for over three weeks, having just been added to the herd and not as yet put into use. All the other bulls had been used an average of once to twice per week before the samples were taken for examination. The other two samples with extremely high counts (numbers 1 and 8) contained large numbers of coliform and pseudomonas organisms as did number 24. All three of these bulls have since been removed from use in the artificial breeding circuits. None of the other samples contained as many as 100 coliform organisms per cc. The average of the logarithms of the plate count of these 15 samples was 22 thousand bacteria per cc. Of 28 semen samples from 7 bulls in the University herd (represented by letters in table 1), the majority contained from 100 thousand to several million bacteria per cc. Only four samples contained as few as ten thousand bacteria per cc. The average of the logarithms of the bacteria in the individual samples was 225 thousand per cc. whereas the arithmetic average of the counts was 2.3 millions. In comparison with the bulls in the artificial breeding circuit, these bulls produced semen containing 10 to 50 times as many bacteria. Whether this is due to frequency and kind of service (natural and artificial vagina) or other factors is not elucidated in this study.

The types of bacteria reported in these samples are only those which occurred in large numbers and not all types present. All the types reported by other workers as frequently found in semen have been found with the exception of streptococci. The organisms occurring most regularly in large numbers were diphtheroids. Hemolytic diphtheroid organisms were isolated from one bull (E) when one series of samples was taken, but they were not found a year later when a second series of samples were studied.

The number of bacteria present in these samples was higher than would have been expected from the reports of a number of workers who considered that semen from normal healthy bulls is almost free from bacteria. *Pseudomonas pyocyaneus* was isolated from five of the nineteen bulls studied. In this connection it should be noted that three of the four bulls dropped from breeding operation of the Central New York Artificial Breeders' Cooperative within the last six months were among the five in the semen of which pseudomonas organisms were present in large numbers. Of the other two bulls, one has since become sterile and the other has a record of 12.1 per cent conception from 91 inseminations by artificial breeding. Gilman (3) considered these organisms as among those of importance in genital infections.

Effect of Cleaning the Bull. In order to determine whether it would be possible to reduce the number of bacteria in semen collected with the artificial vagina, two bulls were cleaned, to the extent of thoroughly washing the underline with soap and water, clipping the hair from about the prepuce, and flushing the sheath with sterile distilled water. When bull (D) was treated in the above manner on alternate weeks, the first sample being col-

lected without cleaning the bull, the counts were as follows: 490, 3, 32, 3 in thousands. The reduction in count by the first cleaning was over a hundred fold and in the second case 10 fold, the count not having reached as high a value one week after cleaning as before any treatment. A second bull (I) which gave semen with a lower count without cleaning than D, did not show as great response to cleaning, the counts on consecutive weeks being 13, 1, 39, 6 in thousands. The bull was cleaned before the second and fourth collection. Here the reduction was about 10 fold, the counts being reduced to about the same number as those obtained when bull D was cleaned.

Preparation of Yolk-phosphate Diluent. It was found during the early course of the experiment that aseptic methods must be practiced in order to produce a yolk-phosphate diluent without undue contamination. To produce such a diluent, fresh eggs were obtained from a pullorum-free flock and immersed in normal NaOH or 70 per cent alcohol to sterilize the shell (Bryant and Sharp (2)). The eggs were then broken and the yolk removed, using sterile glassware, and mixed with sterile buffer. By using these precautions yolk-phosphate diluents with very low bacterial counts, could be consistently produced. Thus, of 21 samples of diluent so prepared, 17 had counts between 0 and 15 bacteria per cc., 2 of 150 and 200, and 2 had counts of 1,000,000 and 7,000,000. The last two samples mentioned may have been contaminated by the equipment or the buffer, for immediately after they occurred all the material was sterilized with the result that 12 consecutive samples were prepared during the following month with no count over 200. Four samples prepared with unsterilized buffer had counts ranging from 600,000 to 4,000,000, and two samples prepared with yolk from store eggs without sterilizing the shell or the buffer had counts of 24,000,000 and 166,000,000. As will be shown below, semen-yolk-phosphate mixtures containing such large numbers before storage often had counts in the billions after storage for several days. The yolk-phosphate diluent may be the important source of large numbers of bacteria in stored semen samples if care in preparation is not exercised.

Growth of Bacteria in Semen During Storage. The following studies were performed to investigate the growth of bacteria in undiluted or diluted semen during storage.

In one experiment 4 ejaculates were each divided into 3 parts and stored undiluted, in SGC–2 diluent and in yolk-phosphate diluent at 5° C. and 10° C. Samples from each treatment were examined after 4 and 8 days of storage. The data are summarized in table 2. It can be seen that the yolk-phosphate diluent was an excellent medium for bacterial growth as evidenced by the high count after storage for 8 days at 10° C. The bacteria grew least rapidly in the undiluted semen. As would be expected, there was considerably more bacterial growth at 10° C. than at 5° C. In fact, at the lower tem-

TABLE 2

Effect of storage temperature and diluent on bacterial growth (averages from 4 ejaculates)

Treatment	Bacteria per cc. in thousands after storage				
	5° C.			10° C.	
	0 days	4 days	8 days	4 days	8 days
Undiluted	190	420	400	430	740
Yolk-phosphate	200	250	520	600	12,000
SGC-2	100	130	80	310	2,600

perature there was not enough bacterial growth to effect changes in the medium. Therefore 5° C. or lower is preferable as a storage temperature in order to hold the bacterial growth to a minimum, especially since it has been shown by Willett (18) that storage at 5° C. preserves the viability of the sperm longer than higher storage temperatures.

During the course of Willett's study on the preservation of semen, other observations were made in regard to the growth of bacteria in semen-yolk-phosphate mixtures stored under different conditions. A comparison was made to determine if covering the diluted semen samples with a layer of mineral oil would reduce the growth of bacteria when they were stored for 4 and 8 days as had been indicated by certain preliminary observations. The data, from counts in 9 ejaculates, summarized in table 3 indicated that there was not enough difference between the two treatments to be of practical importance. Also, during the course of these bacteriological investigations several ejaculates were stored in the yolk-phosphate diluent in which the buffer was not sterilized. It was noted that the bacterial counts of such samples during storage reached numbers far exceeding those samples made with sterilized buffer. A summary of these counts is presented in table 3 for comparison with the counts of other uncontaminated samples. Since some workers in the field of artificial insemination recommended temperatures lower than 5° C. for semen storage, samples for 7 ejaculates were stored at both 5° and 1° C., and the bacterial counts compared after 4 and 10 days of storage. It can be seen (table 3) that there was no appreciable difference in numbers of bacteria after storage at the two temperatures.

During these studies of bacterial growth during storage, observations of spermatozoon motility and pH measurements were also made concurrently. The data show no definite relationship between the numbers of bacteria present and the motility of the spermatozoa or pH of the semen during storage. However, because we were largely successful in bacteriological control there were only a few samples in which enough bacteria developed to cause such changes.

The bacteria from bedding, manure, soil, unsterilized glassware, etc., would include *E. coli*, bacilli, and staphylococci. These are the types which

TABLE 3

Bacterial growth during storage in semen yolk-phosphate diluent

Sample pairs	Treatments	Bacteria per cc. (in thousands)					
		Before storage		After storage			
				4 days		8 days	
		Average	Range	Average	Range	Average	Range
No. 9	Mineral oil, 5° C.	582	1–2,550	293	2– 2,100	426	6– 1,300
	No mineral oil, 5° C.	"	"	452	3– 3,550	533	4– 2,250
6	Diluent contaminated, 5° C.	2,490	170–5,500	165,000	100– 580,000	2,910,000	100– 7,150,000
	" " , 10° C.	"	"	464,000	100–2,040,000	12,000,000	175,000–28,500,000
7	5° C.	367	1–1,350	55	3– 105	341	4– 1,000
	1° C.	"	"	47	2– 145	352	2– 1,150

can be eliminated or materially reduced in number by the methods outlined above, and are the types which grow readily at storage temperatures. Therefore, if contamination by these types can be prevented, little bacterial growth will take place in the semen or semen-yolk-phosphate mixture during storage. On the other hand, it appears that the diphtheroids are always present in semen in variable numbers no matter what precautions are taken. However, this type does not grow at the usual low storage temperatures, and therefore, does not become numerous during long storage. In a number of samples pseudomonas organisms were present in large numbers. Since they grow at low temperatures, one would not be able to eliminate growth of these bacteria by using such temperatures.

Germicidal Action of Semen. In the storage experiments above reported it was observed that the number of bacteria decreased during storage in some cases. The question arose as to whether semen had a bactericidal action. Several experiments were performed to see if a bacteriacidal effect could be demonstrated at storage temperatures.

Semen from each ejaculate was divided into two parts. One part of the semen was stored untreated, while the other was inoculated with a culture of *Escherichia coli.* SGC–2 diluent inoculated with *E. coli* served as a control. The samples were stored between 5° and 7° C. without mineral oil in 25 cc. test tubes plugged with cotton. At the start of the storage period the counts were made every one-half or one hour, but the intervals were lengthened with the progress of the storage period. In three studies thus made at these low temperatures, there was no evidence of any germicidal action.

During these experiments frequent observations of spermatozoon motility were also made with rather startling results. It was found in all three experiments that the motility of the spermatozoa in the inoculated samples was markedly superior to those in the untreated semen when diluted and examined after storage. In one experiment a sample of semen was inoculated with coli killed by heating to compare with the other treatments. The spermatozoa in the sample showed no greater motility than the untreated semen.

SUMMARY

1. The bacterial count on 43 ejaculates collected from 19 bulls by means of an artificial vagina ranged from 1,000 to 22,000,000 per cc.

2. It was found that by douching the sheath and washing the underline, if the bull was dirty, the number of bacteria in semen could be markedly reduced.

3. Almost sterile yolk-phosphate diluent was consistently produced when fresh eggs from healthy hens were used and when aseptic methods were employed in the preparation of the diluent. Under other conditions the

diluent may be responsible for the addition of large numbers of bacteria to semen samples.

4. Bacterial growth during storage was held at a minimum by storing at 5° C. or lower.

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PSEUDOMONAS PUTREFACIENS IN DAIRY PLANT EQUIPMENT*

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In outbreaks of putrid butter the sources of the causative organisms undoubtedly vary. Some outbreaks have been traced to water used to wash the butter or equipment (1, 3), while in other instances water has not been involved. *Pseudomonas putrefaciens* commonly is responsible for the putrid defect in salted butter (1, 2), at least in certain areas. It is sensitive to heat, being killed in 2 minutes at 61.7° C. (4), so that it probably never survives pasteurization. A source other than water which could contribute the organism to pasteurized cream or to butter is dairy plant equipment. Attempts to isolate *Ps. putrefaciens* from such equipment are reported herein.

METHODS

Frequently, bacteriological examinations of dairy plant equipment have involved rinsing with sterile water and then culturing the water. In the case of churns this is unsatisfactory because many organisms are between staves, at junctions of ends and staves, around bolts, etc., from which they are dislodged only when there is considerable strain on the churn, for example, when the butter breaks or when it is worked. In these locations the organisms are protected during the attempts to sterilize the churns, and their multiplication continues to supply organisms for the contamination of one lot of butter after another. Much the same situation probably exists with certain other equipment, especially leaky vats.

In the isolation studies reported herein such materials as curd and wood from various points in the equipment were smeared directly, and also after enrichment in litmus milk at 3° C. for varying periods, on a special medium (4) consisting of: gelatin 4 per cent, proteose peptone 2 per cent, dipotassium phosphate 0.1 per cent, ferric ammonium citrate 0.05 per cent, agar 1.5 per cent and water to make 100.0 per cent. On this medium *Ps. putrefaciens* grows relatively well at 21° C. and gives colonies with a rather characteristic appearance. Colonies suggesting *Ps. putrefaciens* were purified and enough of the characters studied to determine whether the organisms belonged to this species.

RESULTS

In some instances *Ps. putrefaciens* was obtained from dairy plant equipment and in other instances isolation attempts failed. Examples of both successful and unsuccessful attempts are as follows:

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Example 1. In a plant making butter that kept satisfactorily in normal marketing channels, an occasional churning became putrid in 5 to 7 days in keeping quality tests at 21° C. Pasteurization of the cream appeared to be efficient, and the wash water was chlorinated. Materials were collected from various parts of two of the several churns in the plant by loosening the shelves, removing bolts, etc.

With churn 1, *Ps. putrefaciens* was recovered by direct smears from between the end of a shelf and the iron cover plate and from wood near a bolt; it was recovered by enrichment from three of four points near bolts; and it was not recovered from five points, including the buttermilk drain, around the sight glass, under a bolt head and on the brace (two points) under a shelf. With churn 2, the organism was recovered directly from the end of the shelf and from under the shelf but not from two other points near the shelf; enrichment did not detect additional positive samples.

Microscopic examinations of the original material indicated that micrococci, gram-positive rods, gram-negative rods, yeasts and molds were present, often in large numbers; these general types of organisms also were evident on the plates.

Example 2. Some months after the collection of the samples referred to in example 1, the spoilage of butter in the keeping quality tests no longer occurred in the plant. Because of its general condition it was necessary to replace an end in one of the churns and during the operation six samples were obtained from around bolts, under the sight glass and at the junction of shelves and ends. None of the samples yielded *Ps. putrefaciens,* either directly or by enrichment. All the plates were heavily overgrown with various microorganisms.

Example 3. A coil vat in a milk plant was discarded because of leaks which permitted liquid to soak into the insulation. Within 24 hours of the last use of the vat, holes were cut through the outer wall and samples of the insulation collected. Near the leaks the insulation was soaked with water and had a very objectionable putrid odor, while at some distance from the leaks it was moist and the odor was somewhat putrid.

Ps. putrefaciens was isolated by direct smears from four of the seven samples but was not obtained by enrichment from any of them. Three of the four positive samples were from portions of the insulation that were very wet, while one was from a portion that was only moist.

After the insulation had dried rather completely over a period of 5 weeks, eight additional samples were collected. Cultures of these, both without and with enrichment, failed to show *Ps. putrefaciens,* the plates being largely overgrown with aerobic spore-forming bacteria.

Example 4. A churn barrel was taken out of a plant because the general condition, particularly of the roll, made repairs inadvisable. The plant had not experienced the putrid defect; however, the butter was consumed

quickly and keeping quality tests were not being run. Since the barrel was to be used for holding water it could not be dismantled and only the roll and one shelf were removed. Material was collected from one end of the roll, from one end of the shelf, from around the shelf support and from around bolt heads.

Ps. putrefaciens was not detected on plates smeared with the different samples, either directly or after enrichment. However, the plates were heavily overgrown with various microorganisms, including micrococci, spore-forming bacteria, gram-negative rods, yeasts and molds.

Example 5. A plant experiencing a serious outbreak of putrid butter quickly brought the difficulty under rather complete control as far as spoilage in commercial channels was concerned. However, many churnings continued to show spoilage after 5 or 6 days in keeping quality tests at 21° C. Various procedures, such as chlorination of the water, unusually high pasteurization of the cream, etc., failed to prevent the spoilage in the keeping quality tests. The barrel of the older of the two churns in the plant was replaced and about 15 inches of one end of the barrel was cut off and sent to the laboratory. Over a period of several days, 67 samples were collected from various parts of the end which was completely broken up in the process.

Ps. putrefaciens was recovered directly from 13 of the samples; 8 of these were from points at the junction of staves and the end of the churn, 2 from between boards in the end of the churn, 2 from under the sight glass and 1 from wood near a bolt. After enrichment the organism was obtained from 3 additional samples, 2 being from points at the junction of staves and the end of the churn and 1 from between boards in the end of the churn. In some instances *Ps. putrefaciens* made up a rather large percentage of the colonies on the plates smeared directly with a sample. The flora of many of the plates also included micrococci, spore-forming bacteria, gram-negative rods and occasionally some yeasts and molds.

DISCUSSION

The isolation of *Ps. putrefaciens* from various points in churns and from the insulation of a leaky vat indicates that equipment may be a source of the organism in dairy products. Evidently *Ps. putrefaciens* survives the cleaning and heating of equipment due to protection afforded in crevices, cracks and joints. The churns from which *Ps. putrefaciens* was isolated were reported to have received thorough and regular cleaning.

The fact that plates smeared with material from churns not yielding *Ps. putrefaciens* often were heavily seeded with other species, many of which were not heat resistant, is additional evidence that conditions in certain churns may be favorable for *Ps. putrefaciens.* It also suggests that equipment may yield other organisms capable of causing defects in dairy products.

The flora of certain plates smeared with material from churns definitely

resembled the flora of plates prepared from putrid butter. Such plates, regardless of whether or not *Ps. putrefaciens* is present, usually contain micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds. This suggests that with defective and highly contaminated butter much of the contamination may originate in the churn.

SUMMARY

Ps. putrefaciens was isolated from churns and from the insulation of a leaky vat. At certain points the organism was present in considerable numbers. In addition to *Ps. putrefaciens,* micrococci, spore-forming bacteria, gram-negative rods and often yeasts and molds were present.

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OXIDIZED FLAVOR IN MILK. IX. THE EFFECT OF THE QUALITY OF HAY AND EARLY STAGE OF LACTATION ON THE CAROTENE CONTENT OF BUTTERFAT AND ON THE ASCORBIC ACID CONTENT OF THE MILK AND THEIR RELATIONSHIP TO THE DEVELOPMENT OF METAL-INDUCED OXIDIZED FLAVOR*

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One of the earliest workers to report a seasonal variation in oxidized flavor was Mattick (11) who in 1927 reported that oiliness in milk appeared in autumn, winter and spring, but never in the summer. Kende (10) recognized this fact and as a result of his work concluded that green feed contained some substance or substances which when fed to the cow protected the milk against the off-flavor. Since that time numerous workers have observed the difference in the susceptibility of winter and summer milk to oxidized flavor. Stebnitz and Sommer (15) found that when cows received grass as part of their ration, the butterfat became less saturated and more susceptible to oxidation. However, it appeared that protective substances in the milk prevented the development of oxidized flavor. Likewise, it is generally agreed that green feeds yield a more stable flavored milk as compared to milk produced on dry feed.

The nature of these inhibiting substances carried in green feed has been the object of extensive investigations. Anderson and co-workers (1, 2) were some of the earliest investigators to show the effect of carotene on the milk flavor. Their work showed that carrots or machine-cured alfalfa fed to cows would eliminate oxidized flavor in their milk. This they correlated with the carotene content of the feed. Anderson, Hardenbergh and Wilson (2) obtained far more effective results in the elimination of oxidized flavor by feeding 8 pounds of carrots in the daily ration than by feeding a cod-liver oil concentrate containing 500,000 U.S.P. units of vitamin A. Whitnah and co-workers (17, 18) and Beck, Whitnah, and Martin (4) report that a carotene supplement quickly corrected the tendency for oxidized flavor to develop spontaneously. In addition they found oxidized flavor more prevalent in milks that were below the breed average in fat color intensity. There were, however, samples low in color which did not develop oxidized flavor. Likewise, it was noted that the color of the fat was not increased for some time

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after the flavor was improved. These workers (18) report that low-carotene intake levels were not the only factor determining the tendency for milk to develop oxidized flavor as shown by the fact that the five lowest carotene intakes per kilogram of body weight among cows producing milk that kept a good flavor were below the three highest intakes among these cows producing milks which developed oxidized flavor.

Brown, Vanlandingham, and Weakley (6) found that a carotene supplement added to the ration rendered the milk less susceptible to metal-induced oxidized flavor. Likewise, the supplementing of a low-carotene ration with ascorbic acid produced similar results. Garrett, Tucker, and Button (9) found a positive correlation between color and flavor and between ascorbic acid and flavor. They concluded that high carotene and high ascorbic acid are coincidental to and help to preserve good flavor in milk. Garrett, Arnold, and Hartman (7) reported that alfalfa silage is almost equal to spring pasture in putting yellow color into milk and is equal to or better than pasture in producing milk of fine flavor and high resistance toward the development of oxidized flavor.

Brown, Thurston, and Dustman (5) found that feeding of one quart per animal per day of either tomato or lemon juice to cows on dry feed reduced the susceptibility of the milk to oxidized flavor. They attributed this effect to ascorbic acid in the feed and observed a similar tendency when pure crystalline ascorbic acid was fed at the rate of ½ gram daily. Nelson and Dahle (12) added orange and tomato juice directly to the milk and observed a slightly greater protective effect than could be accounted for on the basis of their ascorbic acid values.

Garrett, Tucker, and Button (9) obtained information based on the average flavor scores which showed that for a decrease in ascorbic acid there was also a corresponding decrease in flavor score. The apparent critical point of the relationship of ascorbic acid to good flavor was found to lie between 15 and 18 mg. of ascorbic acid per liter of milk. Garrett, Arnold, and Hartman (7) reported that feeding grass silage had a greater stabilizing effect on ascorbic acid in milk than corn silage or beet pulp. This stabilizing effect tended toward milk of better flavor.

It has been shown that feeding carrots or machine-cured alfalfa hay will render milk non-susceptible to oxidized flavor and that both carotene and ascorbic acid in the feed play a role in the susceptibility of the milk to the flavor. Since the carotene in the butterfat is affected by the carotene in the feed, and since good quality hay is known to contain relatively large amounts of carotene, it appears that there should be a relationship between the quality of hay and the susceptibility of the milk to oxidized flavor. Since many producers who have difficulty with oxidized flavor purchase hay, it seemed desirable to know if the feeding of very high quality alfalfa hay would render the milk non-susceptible to metal-induced oxidized flavor. Accordingly, the following experiment was planned and conducted.

EXPERIMENTAL

For use in this experiment, eight Jersey cows, whose milks were susceptible to oxidized flavor when contaminated with copper, were selected and placed on a ration low in carotene. The feed consisted of a grain mixture of 100 lb. ground oats, 100 lb. wheat bran, 15 lb. cottonseed meal, 3 lb. salt, and 2 lb. of steamed bone meal, with different quality alfalfa hay and beet pulp as the roughage.

Three quarts of milk were collected from each cow at the morning milking on the first three consecutive days each week, and carotene, ascorbic acid, and flavor determinations were made. The ascorbic acid was determined on the individual samples of raw milk, as soon as possible after milking, by titrating as suggested by Sharp (16). The remaining milk was pasteurized in bottles. Following pasteurization and cooling, four one-half pint samples were prepared containing none, 0.5, 1.0. and 1.5 parts per million, respectively, of copper from a copper sulphate solution. These samples were then stored in ice water for three days, after which they were scored for flavor by at least three judges familiar with oxidized flavor. The remainder of the milk was prepared for carotene analysis by gravity separation of the cream followed by churning. Before churning, the cream from each of the three days was composited so as to make one churning and one analysis for carotene per cow per week. The butter thus obtained was melted and centrifugalized in Hart's casein tubes in an electrically heated centrifuge for 15 minutes, after which the clear, liquid butter oil was decanted into a clean, dry jar. The carotene analyses were made according to the method of Baumann and Steenbok (3) modified by Rogers and associates (14). After a 5½-week preliminary period on the regular herd ration the animals were changed to the experimental depletion ration for a period of four weeks. This ration consisted of the grain mixture already described, and eight pounds of brown alfalfa hay which still retained its leaves, together with 12 pounds of dry beet pulp.

At the end of the depletion period the animals were divided into 2 groups which were approximately equal in intensity of oxidized flavor. One group was continued on the brown hay roughage while the other group was changed to bright green, leafy, alfalfa hay. The hay given both groups was increased from 8 to 12 pounds per animal per day. Special care was taken to select hay of equal leafiness in both types of hay. The brown hay had an average carotene content of 5.8 mg. of carotene per kilogram whereas the green hay had an average carotene content of 43.0 mg. of carotene per kilogram. After two weeks on this ration the cows that were receiving the bright green hay were given hay *ad lib.* for three weeks and then were supplemented with 2 pounds of alfalfa leaf meal (carotene content 4.9 mg. of carotene per kilogram) daily. The purpose of this supplement was to increase the amount of alfalfa and carotene in the ration. Unfortunately, the amount of caro-

tene in the leaf meal was lower than expected and therefore the carotene supplement was not as great as was intended. However, there was a marked reduction in the intensity of the oxidized flavor of the milk.

After the three-week period in which the cows received leaf meal supplement, there was a readjustment period of three weeks followed by pasture for one week, after which time the experiment was discontinued. The cows

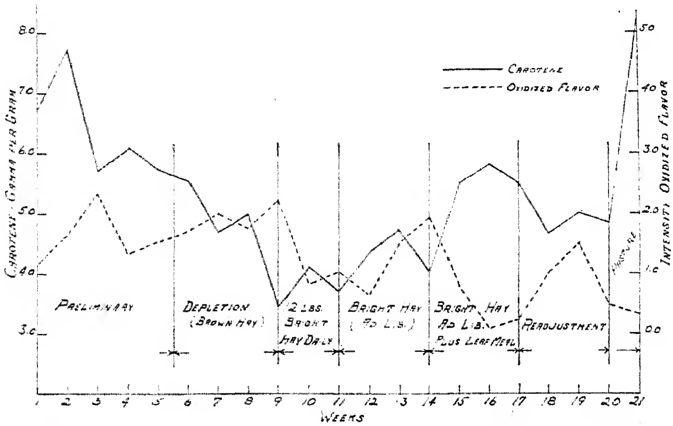

FIG. 1. The relationship between carotene and metal-induced oxidized flavor in the milk produced by cows on bright, green, alfalfa hay.

that received the brown hay were continued on the same basis except that they received no alfalfa leaf meal supplement. These animals were on pasture one week before the experiment was discontinued. The results of this experiment are shown graphically in figures 1 and 2. Figure 1 gives the average of the results obtained with the group on bright green hay while figure 2 gives the average results obtained on brown hay during the same periods.

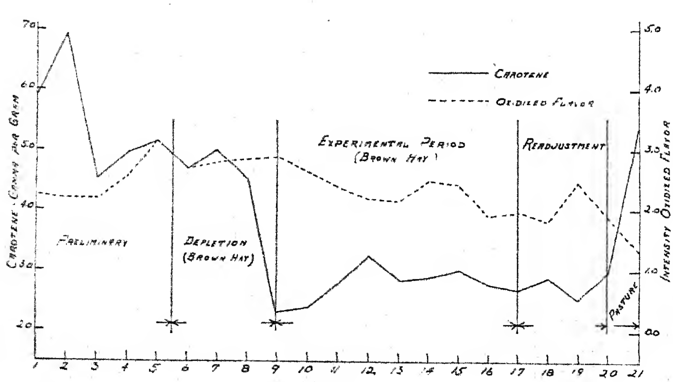

FIG. 2. The relationship between carotene and metal-induced oxidized flav[or] produced by cows on a low-carotene ration receiving brown hay.

The results shown in figure 1 indicate that there may be a relationship between the carotene in the butterfat and the intensity of oxidized flavor developed, but this relationship is not very clear. During the first nine weeks there was a great reduction in the carotene of the butterfat, but there was no significant increase in the susceptibility of the milk to oxidized flavor. When the ration was supplemented with alfalfa leaf meal even though relatively low in carotene *per se,* there was an increase in the carotene of the butterfat and a corresponding decrease in the intensity of the oxidized flavor until it was almost eliminated. It may be observed, however, that the carotene in the butterfat, during the period when the ration was supplemented with alfalfa meal and practically devoid of oxidized flavor, was not as high as at the beginning of the experiment, when the oxidized flavor was relatively strong.

Figure 2 shows the relationship between the carotene content of the fat and the intensity of oxidized flavor of milk produced on brown hay. Here there seems to be an absolute lack of relationship, since the carotene content of the milk was greatly reduced and maintained at a low level for some time. It might be expected that the intensity of the oxidized flavor would be increased and that possibly some spontaneous oxidized flavor development might occur. However, examination of the data reveals that, contrary to the expected results, we find if anything a slight reduction in the intensity of the oxidized flavor developed.

There was no apparent relationship between the ascorbic acid content of the milk and the susceptibility of the milk as influenced by the quality of hay.

STAGE OF LACTATION

While the experiment on the quality of hay was in progress it was noticed that certain animals which had recently calved were producing milk relatively high in carotene but nevertheless with a strong oxidized flavor. It was decided to study the effect of early stage of lactation on the development of this flavor. Accordingly, samples were taken from nineteen cows which had just freshened on the herd ration. Carotene, ascorbic acid, and metal-induced oxidized flavor development in their milks were studied. All determinations were made in the same manner as in the previous experiment. The results obtained from the 19 cows studied are shown in figure 3. Examination of these data reveals that there was a marked decrease in the carotene of the butterfat for the first three weeks of lactation, after which it remained fairly constant for the next eight weeks. The intensity of the oxidized flavor developed followed a very similar curve and almost paralleled the carotene curve. In contrast to the results of Rasmussen *et al.* (13), it was found that the ascorbic acid content at the beginning of lactation was unusually low and increased gradually until it reached a maximum level at about eight weeks following parturition. These results verify the work of Whitnah and Rid-

dell (19), who found an average increase of 10 per cent in vitamin C concentration from the first to the second month of lactation. In this experiment there appeared to be an inverse relationship between the amount of ascorbic acid in the milk and the intensity of the metal-induced oxidized flavor.

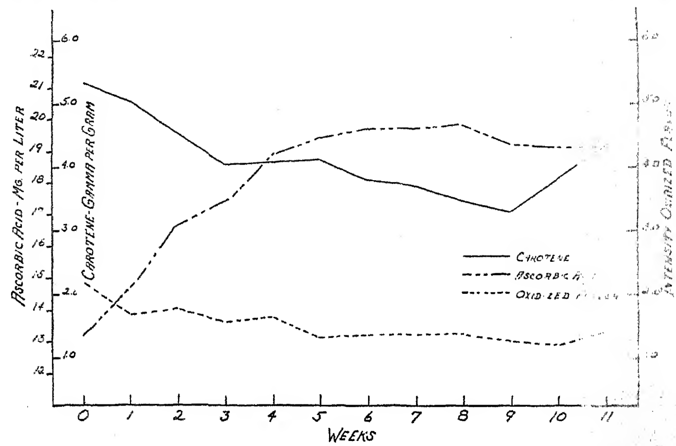

FIG. 3. The relationship between the carotene content of the butterfat, the ascorbic acid content of the milk, and the intensity of metal-induced oxidized flavor, as shown during the early stages of lactation.

Since there was no apparent relationship between the ascorbic acid content of the milk and the intensity of the oxidized flavor depending upon the quality of the hay, it was decided to arrange all values obtained in groups based on the intensity of the oxidized flavor. Figure 4 shows the relationship of ascorbic acid to intensity of oxidized flavor as shown by 580 observa-

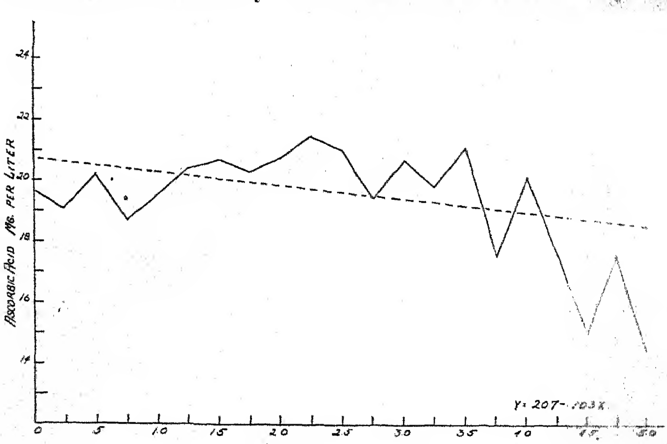

FIG. 4. The relationship between the ascorbic acid content of the milk and the intensity of metal-induced oxidized flavor.